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**Swimming behaviour and prey perception
in the calanoid copepod
*Clausocalanus furcatus***

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Abstract

Small planktonic copepods (≤ 1 mm) play a pivotal role in the functioning of marine ecosystems. Among them, the calanoid *Clausocalanus furcatus* is one of the dominant species in epipelagic waters of tropical and sub-tropical oceans. Its swimming motion is rather unique among planktonic copepods, being characterized by a continuous looping movement performed at very high speed.

The aim of this PhD thesis was to investigate some aspects of the swimming behaviour of *C. furcatus* adult female that could shed light on its small-scale interactions with the surrounding environment. In particular, the work was focused on the mechanisms regulating the perception and capture of prey items, in order to understand the success of this species in oligotrophic regions. To this purpose, four different approaches have been utilised:

- the numerical characterization of the trajectories performed by freely swimming *C. furcatus* adult females in presence of dinoflagellate cells and in association with capture events;
- the reconstruction of the perception area and of the mechanisms involved in the capture of prey items;
- the morphological investigation of the sensory structures present on the first antennae, which bear mechano- and chemo-sensors involved in the perception of external cues;
- the evaluation of the sensory performance of the mechanoreceptors on the first antennae by means of electrophysiological experiments.

The results of these investigations concur in depicting a coherent scenario. In *C. furcatus*, the modalities of detecting and capturing prey seem to be forged to efficiently exploit food particles when they are grouped in small patches, and are aimed at maximising the success of *C. furcatus* in oligotrophic environments.

Chapter 1

The world of copepods

1.1 The importance of copepods in aquatic environments

On Earth, aquatic ecosystems are inhabited by a variegated universe of microscopic organisms. They were discovered for the first time by van Leeuwenhoek (1677), but they were named "plankton" only later by Hensen (1887). This micro-universe is made of both plants (phytoplankton) and animals (zooplankton) belonging to several different taxa, and it occupies a wide size spectrum, ranging from 10^{-6} (μm) to 10^1 m (Sieburth *et al.*, 1978).

Zooplankton consists of the majority of zoological phyla, from protozoa to small fish, but is numerically dominated by copepods, the small-size crustaceans commonly referred to as "the insects of the sea" (Huys & Boxshall, 1991). They are the most abundant metazoans of the world (Hardy, 1970; Wiebe *et al.*, 1992) outnumbering insects, which are however characterized by higher diversity (Humes, 1994; Mauchline, 1998). Copepods represent up to 90-97% of the total biomass of marine zooplankton (Bradford-Grieve *et al.*, 1999), also being abundant in freshwaters (Huys & Boxshall, 1991).

The class Copepoda comprises nine orders: Platycopioida, Calanoida, Misophrioida, Harpacticoida, Monstrilloida, Mormonilloida, Gelyelloida, Cyclopoida, Siphonostomatoida, (Boxshall & Halsey, 2004). 11.500 copepod species are currently known (Humes, 1994), even though the actual number may reasonably be higher (Huys & Boxshall, 1991). Free-living copepods have colonised all the available habitats (pelagic, benthopelagic, hyperbenthic, benthic, cryptic, and subterranean). Some species dwell in association with other organisms (either plants or animals) (Huys & Boxshall, 1991). Moreover, copepods occur from the deep-sea trenches (Wolff, 1960) to the Nepal mountains (Löffler, 1968), occupying a wide vertical range (approximately 20 Km: Huys & Boxshall, 1991).

Besides their numerical and geographical importance, freely swimming copepods are primary actors in the functioning of pelagic ecosystems. Copepods provide the link between lower (e.g., phytoplankton) and higher (e.g., fish) levels (Naganuma, 1996); some planktonic species are also able to prey upon fish larvae (Lillelund & Lasker,

1971). The predation upon phytoplankton provides the energy for metabolic requirements, but determines also the egestion of fecal pellets which, by passive sinking, enhance the vertical fluxes of carbon from the upper layers towards the deep ocean (Fowler & Knauer, 1986). Copepods are responsible for the release of CO₂ (through respiration) and NH₄ (through excretion) as well, thus contributing to sustaining the microbial loop, the recycled production and the biogeochemical fluxes in the oceans.

The role played by copepods in large-scale dynamics is mediated by the behaviour displayed at the individual level. These two scales are in fact intimately correlated: small-scale interactions with other organisms (prey, predators and mates) are regulated by the behaviour of copepods, which in turn affects the patterns observed at larger scales (Dodson, 1996).

The presence of copepods is therefore of paramount importance for the proper functioning of aquatic systems: they represent a key component of the pelagic food web, and their role is fundamental for both ecological and commercial aspects (Bradford-Grieve *et al.*, 1999). It follows that a detailed understanding of their behaviour and ecological role will improve our knowledge of the functioning of aquatic systems *sensu lato*.

1.2 The question of scale

A sound study of copepod behaviour requires the adoption of proper temporal and spatial scales (Turner *et al.*, 1993). Scale influences the physical properties of the environment in which copepods live, as well as the duration of the interactions with other organisms. It is consequently important to utilise appropriate scales and reference frames in the analysis of copepods' behavioural traits.

1.2.1 Observing copepods at their proper scales

Copepods' reduced dimensions represent a strong hindrance to their study and observation. Their body size typically ranges between 0.5 and 5.0 mm (Huys & Boxshall, 1991) with variations occurring between orders, species and developmental stage. In most cases, copepods are barely visible to naked eye. Taxonomical investigations have long been realised through conventional light microscopy, whereas electron microscopy has been developed only relatively recently for more in-depth analysis. These tools are unfortunately not suitable for behavioural studies, for which specific set-ups are needed. These latter must indeed be characterised by high resolution, in order to allow a good visualisation of the animal observed, but must be capable of high frequency rate of visualisation as well, since "behavioral events occur faster than the (human) eye can see" (Turner *et al.*, 1993). In addition, data need to be collected for reasonably long times (Seuront *et al.*, 2004a).

A first breakthrough has been represented by the adoption of cinematographic techniques (e.g., Alcaraz *et al.*, 1980; Rosenberg, 1980; Strickler, 1982; Price & Paffenhöfer, 1984; Strickler, 1985), which permitted the investigation of the feeding mechanisms exploited by different copepods. These observations have generally been performed on tethered copepods using a very high sampling rate, usually of the order of hundreds of frames per second (e.g., Paffenhöfer *et al.*, 1982; Cowles & Strickler, 1983; Price *et al.*, 1983) to follow the movement of the feeding appendages. More recently, the utilization of particle image velocimetry (PIV) techniques has given even more details on the structure of copepod feeding currents (van Duren, 2000).

Nevertheless working on tethered specimens has two main drawbacks. Firstly, the animal may be disturbed by the presence of the tethering device (usually a dog or cat hair, or a fine tin electrical wire), and consequently its responses may not be completely natural. Secondly, constrained specimens are not allowed to swim and therefore it is not possible to analyse their swimming behaviour. All this has prompted the realization of specific video apparatuses by which to visualise and record the tracks described by zooplankters freely swimming in microcosms. This aim however

has been achieved only through the assemblage of sophisticated optical systems. The first recordings have been performed adopting specific illumination techniques and infrared sensitive film (Strickler, 1970 and 1977). Later on, the development of high-resolution TV cameras equipped with appropriate lenses and usually connected to a video cassette recorder for track recording and storing have permitted the analysis of two-dimensional zooplankton swimming trajectories (Wong & Sprules, 1986; Tiselius & Jonsson, 1990; Saiz & Alcaraz, 1992; Tiselius, 1992; Kjørboe *et al.*, 1996; Tsuda & Miller, 1998; Baillieul & Blust, 1999; Broglio *et al.*, 2001; Seuront *et al.*, 2004d). Nonetheless, while terrestrial animals move in a *quasi* two-dimensional world, marine organisms and birds move in a three-dimensional environment (Childress, 1981). Three-dimensional tracks require more elaborate set-ups (Strickler, 1985; Ramcharan & Sprules, 1989; Fields & Yen, 1996; Strickler, 1998; Schmitt & Seuront, 2001; Weber & Van Noordwijk, 2002; Malkiel *et al.*, 2003). Unlike the cinematographic techniques described above, the frequency of sampling of swimming paths is much lower, usually around tens of frames per second (e.g., Strickler, 1998; Mazzocchi & Paffenhöfer, 1999; Nihongi *et al.*, 2004; Uttieri *et al.*, 2004): such values are generally high enough to reconstruct the swimming motion without resolving the movement of the appendages.

The techniques described above have always been utilised in the laboratory, where standardised conditions aimed at minimising any experimentally-induced behaviour should be adopted (Buchanan *et al.*, 1982). Despite Carpenter's (1996) criticism on the reliability of microcosm experiments, these approaches provide details which could not otherwise be obtained, and have always been performed considering any possible container-effect. To tackle this criticism, some efforts have also been made in the realisation of video equipment for working *in situ* (Jaffe *et al.*, 1995; McGehee & Jaffe, 1996; Jaffe *et al.*, 1998; Genin *et al.*, 2005). So far, they have proved their reliability in tracking larger zooplankters moving in their natural environment, but it is reasonable to hypothesize that in the near future technological advances will improve

the resolution of these systems, allowing direct observations of copepods as well (see Katz *et al.*, 1999).

Time also represents a scale to be considered with extreme care. Most of the interactions with the surrounding environment and with other organisms (prey, predators or mates) take place over short time scales. For example, the handling of food particles by means of the mouthparts occurs over periods of a few hundred milliseconds (e.g., Paffenhöfer *et al.*, 1982; Price *et al.*, 1983), whereas the initiation of an escape response from an approaching predator takes place in a few ms (e.g., Lenz & Hartline, 1999; Lenz *et al.*, 2000; Buskey *et al.*, 2002; Lenz *et al.*, 2004).

It is therefore only by working at the small scale, both in terms of space and time, that a proper understanding of copepods' behaviour can be obtained. This is an indispensable prerequisite, whose lack may easily result in misunderstanding and wrong conclusions.

In the last decade, the study of zooplankton behaviour has also been bolstered by the development of numerical models, as reviewed by Carlotti *et al.* (2000). Strictly dealing with motion tracks, the simplest model to simulate organism trajectories is the classical random walk (Okubo, 1986; Davis *et al.*, 1991; Yamazaki, 1993; Yamazaki & Haury, 1993; Yamazaki & Okubo, 1995). However, Kareiva & Shigesada (1983) first raised the issue of considering animals' tracks as not purely random, but as correlated random walks. This has been verified for different organisms (as discussed in Uttieri *et al.*, 2005), including reef fish larvae (Codling *et al.*, 2004) and zooplankters (Uttieri *et al.*, 2004). The optimal way to model zooplankton swimming is to couple a numerical description with a behavioural component; this has been performed by Keiyo *et al.* (1994), whose results indicate that this would be the ideal way for a proper modelling of copepod tracks. Probably, future modelling efforts will contribute to the development of this line of research, especially if integrated with direct copepod tracking.

1.2.2 Living at small scales

When a body moves through a fluid, two forces act on them: an inertial force, which maintains the movement, and a viscous one, which instead tends to slow it down (Vogel, 1994). The dimensionless ratio between these two forces is the Reynolds number (Kundu, 1990):

$$Re = \frac{UL}{\nu}$$

where U =body velocity (m s^{-1}), L =body length (m), and ν =kinematic viscosity of the medium ($\text{m}^2 \text{s}^{-1}$), which is the ratio between the density of the medium ρ (kg m^{-3}) and the dynamic viscosity μ ($\text{kg m}^{-1} \text{s}^{-1}$). While ν can be considered fixed for sea water ($\sim 1.4 \times 10^{-6} \text{ m}^2 \text{s}^{-1}$) (Pond & Pickard, 1983), U and L depend on the velocity and on the length of the body considered.

The Reynolds number characterises the nature of the flow. For high Re values ($>10^3$) inertial forces dominate, viscosity is negligible and turbulence is present; the opposite scenario holds for low Re ($<10^0$), where the flow is laminar and turbulent structures are damped by viscosity (Schneider & Moore, 2000). An alternative distinction is the following: at $Re \ll 1$ a Stokesian realm exists, where momentum is instantaneously diffused throughout the fluid and the resistive force on a buoyant body immersed in it is always zero; on the other hand, $Re \gg 1$ characterise the Eulerian regime, in which propulsion depends on the reaction of the fluid to acceleration (Childress, 1981).

In the case of copepods, swimming velocities are between 1 and 1000 mm s^{-1} (Yen, 2000) which, coupled with their range of body sizes (Huys and Boxshall, 1991), indicates that they live over 5 orders of magnitude of Re values (Yen & Strickler, 1996; Yen, 2000). When at low Re , they dwell in environments dominated by viscosity, where the flow is laminar, inertia is negligible and motion stops when propulsion ceases (Vogel, 1994); by contrast, at high Re values copepods' environment is dominated by inertia and motion continues even after thrust has occurred. Therefore copepods can be considered as living at the borderline between

the inertial and the viscous realms (Alcaraz, 1997; Yen, 2000), bridging these two hydrodynamic environments (Nagamuna, 1996). It is worth underlining, however, that copepods experience their surrounding as inertial only when their motion is very fast, as in the case of an escape response (e.g., Singarajah, 1975; Fields & Yen, 1996; Buskey *et al.*, 2002). For typical cruising speeds, instead, copepods can be considered as swimming in the viscous realm; in this latter case, copepods experience water as a sticky fluid, like if a man would swim in a pool of honey (Mann & Lazier, 1996) or in wax (Childress, 1981). This however has not prevented them from adopting a large variety of swimming modalities, describing different and often highly convoluted tracks (e.g., Ponomareva and Suslyayev, 1980; Kurbatov, 1987; Wong, 1988; Mazzocchi and Paffenhöfer, 1999).

1.2.3 Encountering other individuals

Encounters decide the fate of each organism. A copepod will look for prey, in order to fulfil its energetic requirements, and for mates, in order to have offspring and ensure the survival of the species; at the same time, it must also avoid coming across potential predators which may attack it. The interactions between planktonic organisms are crucially dependent on their own sensory system and performance and on their swimming behaviour, as well as on the physical constraints of their surroundings.

In their model, Gerritsen & Strickler (1977) investigated the factors regulating the encounter probabilities between a predator and its prey. In the model, a generic copepod could be considered as either a predator chasing after a prey or as a quarry fleeing from a marauder. For an interaction to be successful (i.e. ending up in an encounter of the two organisms involved), four consecutive and interrelated steps are needed, namely (Holling, 1966):

- 1-encounter
- 2- recognition
- 3- capture

4- ingestion

The first two points are a function of the perceptive performances of both the predator and the prey. Copepods are equipped with both mechano- (e.g., Strickler & Bal, 1973; Landry, 1980) and chemoreceptors (e.g., Griffiths & Frost, 1976; Bundy & Paffenhöfer, 1993), by which to perceive stimuli from the surrounding environment; on the basis of the cue perceived, they can decide which behaviour to adopt. Capture is a function of the feeding behaviour of the animal: it may either entrain the particle in its feeding current (e.g., Koehl & Strickler, 1981; Tiselius & Jonsson, 1990; Visser & Jonsson, 2000; Malkiel *et al.*, 2003), or attack it through a feeding bout (e.g., Tiselius & Jonsson, 1990; Bundy *et al.*, 1998; Doall *et al.*, 2002; Paffenhöfer & Mazzocchi, 2002); it is worth underlining that the same species can switch between one strategy and the other (Kjørboe *et al.*, 1996), depending on the conditions experienced. As last stage, the prey is handled by copepods' mouthparts (e.g., Paffenhöfer *et al.*, 1982) and tasted through the chemoreceptors present on them (Friedman & Strickler, 1975), eventually being rejected if not palatable (e.g., Bundy *et al.*, 1998).

The encounter rate is numerically given by:

$$Z_p = \begin{cases} \frac{\pi R^2 N_H}{3} \left(\frac{\bar{u}^2 + 3v^2}{v} \right) & \text{for } v \geq \bar{u} \\ \frac{\pi R^2 N_H}{3} \left(\frac{v^2 + 3\bar{u}^2}{\bar{u}} \right) & \text{for } \bar{u} \geq v \end{cases} \quad (1.1)$$

where Z_p is the encounter rate of the predator with its prey, R is predator's encounter radius (defined as the distance at which the predator recognises a prey), N_H is the density of prey, \bar{u} is prey population mean speed and v is the velocity of the predator. The analysis of the above reported equations show that the most important factors influencing the interaction are the encounter radius and the velocities of the organisms involved. From the predator's point of view, Z_p will benefit from an increase in R and in the swimming velocity; moreover, Z_p will also depend on the relative velocity of the marauder and its quarry.

As a result, two strategies arise as optimal predatory behaviour: cruising and ambush. In the former case, the predator swims continuously and preys upon prey of all speeds; this brings a higher probability of encounter, counteracting the increased metabolic expenditure due to the incessant motion. Ambush predators, on the other hand, are almost non-moving: their Z_p relies exclusively on their R , and they are specialised at hunting fast-moving quarries. Being motionless, their metabolic requirements are reduced, but as a drawback their rate of encounter will be lower than for cruising predators.

In their computations, Gerritsen & Strickler (1977) did not include the effects of small-scale turbulence on the velocities of both the predator and the prey. This point has first been discussed by Rothschild & Osborn (1988) and soon after modified by Evans (1989). After these authors, equation 1.1 presents a third velocity component, w , accounting for the root-mean-square turbulent velocity, whose presence determines an increase in Z_p values. Not only does small-scale turbulence enhance the velocity differences between the two organisms involved, but it also may represent a way for the organisms to save metabolic energy otherwise expended in active motion. It is worth underlying here however that w has a direct effect solely on the rate of encounter between a predator and its prey, and not necessarily on the ingestions.

The above theoretical discussion for predatory interactions has a general validity and holds for all inter-individual encounters. The model by Gerritsen & Strickler (1977) can be applied to mating as well, the male in the role of a predator looking for its prey (i.e. the female), as discussed by Buskey (1998). The events involved in the process of mate finding are roughly the same of prey searching (Holling, 1966). The difference lies in just the last step which for predation is represented by ingestion, whereas in mating corresponds to copulation.

1.3 The importance of small copepods

As already stressed, mesozooplankton is highly diversified and covers a wide range of dimensions. It is however only in the last 15 years, since the work by Morales *et al.* (1991), that increasing attention is being devoted to the understanding of the importance of small copepods ($\leq 1\text{mm}$) in oceanic epipelagic communities.

Small copepods have been recognised to represent a key component of the plankton community (see Turner, 2004 for a review on this topic). Small copepods constitute a large portion of total zooplanktonic biomass (Dam *et al.*, 1993; Calbet *et al.*, 2001; Hopcroft *et al.*, 2001; Youssara & Gaudy, 2001; Fernández de Puelles *et al.*, 2003). They play a relevant role in grazing (Morales *et al.*, 1991; Turner, 2004 and references therein), link the classical and the microbial food webs (Stoecker & Capuzzo, 1990; Kleppel, 1993; Roff *et al.*, 1995) and are fundamental prey for larval fish and other zooplankton consumers (Øresland, 2000; Turner, 2004 and references therein). Even when not substantially contributing to biomass, as for example in Arctic areas (Hopcroft *et al.*, 2005), small copepods represent up to 25% of zooplankton production. Their sheer abundance can be justified considering their variety in reproductive strategies and in feeding habits, which contribute to their ubiquity and to their survival (Turner, 2004 and references therein).

Notwithstanding their overwhelming abundance, the importance of small copepods has been acknowledged only in relatively recent times primarily owing to the improvement in sampling methods (Krsinic & Lucic, 1994; Gallienne & Robins, 2001). Plankton nets were usually equipped with meshes $>200\text{--}300\text{ }\mu\text{m}$, mainly to avoid clogging due to presence of phytoplankton and because zooplankton sampling was often associated with the collection of fish larvae. As a result, smaller species were systematically undersampled, and their abundance underestimated. This has unavoidably led to a delay in the investigation of their behaviour, resulting in a paucity of literature on this issue compared to that available for larger species, as underlined by Mazzocchi & Paffenöfer (1998).

1.4 The calanoid copepod *Clausocalanus furcatus*

Among small copepods, the calanoid genus *Clausocalanus* (~0.7-1.5 mm total length) is one of the most common and abundant. *Clausocalanus furcatus* (Brady, 1883) (Figure 1.1) is a typical representative of the genus, of which it is the most divergent species from a phylogenetic perspective (Bucklin *et al.*, 2003). Female body is usually 1.1-1.2 mm long, while the male is shorter (~0.83 mm) (Rose, 1933). The feeding appendages are covered with setae and setules, and the mandibles bear pores that are presumably chemoreceptors (Baiano & Mazzocchi, *unpub. data*).

C. furcatus occurs in tropical and subtropical areas of both hemispheres (Frost & Fleminger, 1968), being abundant in oligotrophic environments (Webber & Roff, 1995a; Siokou-Frangou *et al.*, 1997) as well as in eutrophic waters (Mazzocchi & Ribera d'Alcalà, 1995); in addition, it can be found in both coastal areas and open waters. In the Bay of Naples it presents a major peak of abundance in August, with a secondary minor peak in October-November, whereas for the rest of the year the abundances are drastically reduced (Mazzocchi & Ribera d'Alcalà, 1995; Peralba & Mazzocchi, 2004; Ribera d'Alcalà *et al.*, 2004).

When present, *C. furcatus* is constrained to the upper part of the water column (Vives, 1966; Hure & Scotto di Carlo, 1970; Regner, 1976; Scotto di Carlo *et al.*, 1984; Fragopoulou & Lykakis, 1990; Webber & Roff, 1995a and b; Siokou-Frangou *et al.*, 1997; Lopes *et al.*, 1999; Fragopoulou *et al.*, 2001; Paffenhöfer & Mazzocchi, 2003; Lo *et al.*, 2004; Peralba & Mazzocchi, 2004), always dwelling above the thermocline (Paffenhöfer & Mazzocchi, 2003; Peralba & Mazzocchi, 2004). This distributional pattern does not show any nyctimeral (Moraitou-Apostolopoulou, 1971; Sameoto, 1986; Fragopoulou & Lykakis, 1990; Paffenhöfer & Mazzocchi, 2003; Lo *et al.*, 2004) or seasonal variation (Moraitou-Apostolopoulou, 1971; Peralba & Mazzocchi, 2004).

Although its spatial and temporal distributions are well-documented, very little is known about the biology of *C. furcatus*. A pioneering work by Mazzocchi & Paffenhöfer

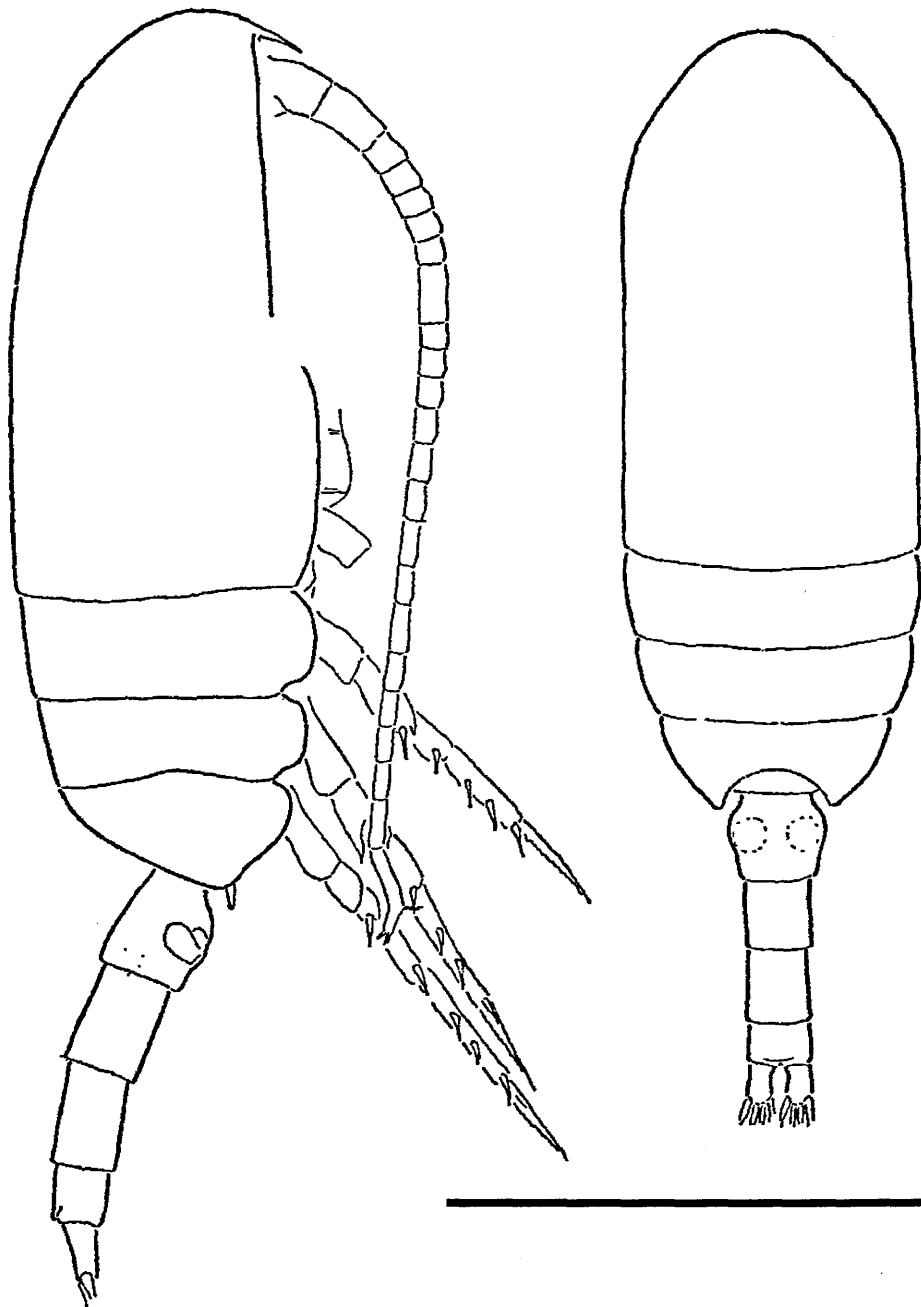


Figure 1.1: camera lucida drawing (right lateral and dorsal views) of an adult female *Clausocalanus furcatus*; scale bar equal to 0.4 mm (modified from Frost & Fleminger, 1968).

(1998) shed light on some aspects of its feeding, reproduction and development. *C. furcatus* spends most of the time (73-100%) moving actively (Mazzocchi &

Paffenhöfer, 1999) by a repetitive looping performed at high speed (10 mm s^{-1} on average), with frequent changes (and often complete reversals) of direction. These loops are sometimes interrupted by short but fast (up to 16 mm s^{-1}) linear displacements; on occasion, these loops alternate with a “hop and sink” swimming or with very fast somersaults (Mazzocchi & Paffenhöfer, 1999). This peculiar swimming motion is unique among planktonic copepods, which usually adopt more conventional swimming modes (see, for example, Mauchline, 1998, page 404); only the large copepod *Pleuromamma* appears to display a relatively similar swimming behaviour (Mazzocchi, *pers. obs.*). It worth noticing that the typical looping motion is maintained by *C. furcatus* nauplii as well, as reported by Björnberg (1972). The distinctive swimming motion of this small calanoid poses a series of questions about the behaviour of the copepod.

This overview on *C. furcatus* draws attention to the marked discrepancy of knowledge between the atypical swimming behaviour of the copepod and its seasonal cycle. The small body size, the typical fast and looping propulsion and the difficulty of rearing *C. furcatus* have prevented a detailed investigation of its small-scale behaviour, the only data having been collected by Mazzocchi & Paffenhöfer (1998 and 1999). A detailed understanding of the ecological role of *C. furcatus* cannot however be gained by looking at its large-scale patterns only, but needs a complementary investigation of the individual-level mechanisms of interaction both with the environment and with other organisms.

1.5 Aim of the thesis

The characteristic natatorial activity of a copepod affects the mechanisms of prey perception and capture. The main aim of this PhD thesis is to provide details about the behavioural and morphological adaptations of *C. furcatus* in the interaction with its prey. The answer to this general enquiry should provide new insight into the small-

scale aspects of the behaviour of this calanoid copepod by investigating the mechanisms by which it perceives and captures food items. On the other hand, the issue raised in the driving question is interdisciplinary in its essence, and requires the investigation and the integration of different aspects related to both the natatory and the sensory performances of *C. furcatus*. On this premise, the experimental work of this thesis has consisted of four different approaches, each focusing on a specific aspect of the swimming and of the sensitivity of the copepod:

1- the numerical characterization of the trajectories performed by freely swimming *C. furcatus* adult females both during normal swimming and in association with capture events;

2- the reconstruction of the perceptive field and of the mechanisms involved in the capture of prey items;

3- the morphological investigation of the sensory structures present on the first antennae of *C. furcatus*, which bear mechano- and chemo-sensors involved in the perception of external cues;

4- the evaluation of the sensory performance of the mechanoreceptors on the first antennae by means of electrophysiological experiments.

In the following chapters, details will be provided about the methods adopted and the results obtained for each of the previously listed approaches. Chapter 2 will focus on the numerical characterisation of the tracks described by *C. furcatus* in the presence of its prey, either associated to a capture event or not. The proper description of swimming paths provides insight into the strategies adopted by the animal and on the possible modifications triggered by the presence of the prey. Chapter 3 will be centred upon the investigation of the geometrical arrangement of successful captures, with the aim of understanding which are the predatory mechanisms adopted by *C. furcatus*. Chapter 4 will deal with the role of the first antennae in the detection of stimuli from the surrounding environment. This task will be accomplished by analysing the sensory structures present on copepod's first antennae and by recording its neurophysiological response to a mechanical stimulus

mimicking an approaching quarry. Finally, Chapter 5 will be the suitable venue to merge the different results obtained, each representing the tessera of a mosaic accounting for the behaviour of the copepod. The interdisciplinary and multi-perspective approach utilised in this thesis work represents an innovative contribution in the study of zooplankton behaviour. Each approach utilised focuses on a specific aspect of the swimming and of the prey perception, but it is only through their integration that a comprehensive picture of the small-scale behaviour of *C. furcatus* can be built. This will provide new insight into the mechanisms by which the copepod can efficiently exploit the scant resources available in a food-dilute environment, leading to its success in oligotrophic environments.

Chapter 2

**Characterization of the swimming
trajectories of
*Clausocalanus furcatus***

2.1 Swimming trajectories as a behavioural key

Planktonic organisms are by definition passively buoyant (Hensen, 1887), only drifting with the motion of water masses. Actually plankters, and more particularly zooplankters, are able to actively displace themselves and to control their horizontal (Kaartvedt, 1993) and vertical (Genin *et al.*, 2005) position in the water column in spite of net water movements.

Copepods typically search for food and mates, while at the same time they try to avoid hazardous encounters with potential predators. Motion can be considered a basic adaptative trait and consequently a relevant source of information on the biological and behavioural responses of animals to variable environmental conditions (Table I). It is also a key aspect for understanding both the autoecology (Strickler, 1977) and the niche separation (Strickler, 1985; Paffenhöfer & Mazzocchi, 2002) of species.

Zooplankton swimming trajectories therefore represent a synthetic descriptor of their own behaviour, as a consequence of their specific responses to both internal and external stimuli. This results in morphologically different (and often highly convoluted) routes, with a large variety of swimming modes (see, for example: Ponomareva & Suslyayev, 1980; Kurbatov, 1987; Buskey *et al.*, 1993; Mazzocchi & Paffenhöfer, 1999; Uttieri *et al.*, 2004). Such behavioural variability is not limited to adult organisms only, but is indeed displayed by naupliar and copepodid stages as well (Paffenhöfer *et al.*, 1996; Titelman & Kiørboe, 2003a and b).

The analysis of zooplankton natatory tracks plays a crucial role in the comprehension of the interactions, occurring at the scale of the individual, with the surrounding environment. There are however two major hindrances hampering such investigation. The first is represented by the need for appropriate video and optical set-ups with which to visualise and record motion, which naturally occurs in a three-dimensional reference frame. The second is the necessity of finding suitable numerical tools with which to characterise the tracks properly and exhaustively. So far, zooplankton tracks have been analysed mainly by means of simple metrics, such as velocity (e.g., Strickler, 1970; Porter *et al.*, 1982), turning rate (e.g., Dodson *et al.*,

Table 1: literature overview, arranged in chronological order, of zooplankton swimming behaviour as influenced by different variables.

Author	Variable Investigated	Zooplanktonic Organism
Baylor & Smith (1953)	Light	<i>Daphnia</i>
Smith & Baylor (1953)	Light	<i>Daphnia</i>
Strickler (1970)	Light	<i>Cyclops</i>
Katona (1973)	Mating	<i>Eurytemora affinis</i> , <i>Eurytemora herdmanni</i> , <i>Pseudodiaptomus coronatus</i>
Singarajah (1975)	Light and turbulence	Larvae of <i>Polydora ciliata</i> , <i>Acartia clausi</i> , <i>Centropages hamatus</i> , <i>Temora longicornis</i> , <i>Tisbe furcata</i> , larvae of <i>Portunus holsatus</i> <i>Calanus pacificus</i> , <i>Pseudocalanus</i> sp. <i>Calanus finmarchicus</i>
Griffiths & Frost (1976)	Mating	<i>Mesocyclops edax</i>
Haury et al. (1980)	Fluid mechanical signal	<i>Centropages typicus</i>
Williamson (1981)	Prey densities	<i>Pseudocalanus minutus</i>
Cowles & Strickler (1983)	Prey densities	<i>Daphnia magna</i>
Buskey (1984)	Presence of prey	<i>Limnocalanus macrurus</i> , <i>Senecella calanoides</i> , <i>Episcura lacustris</i>
Young et al. (1984)	Light	<i>Oithona davisae</i>
Wong & Sprules (1986)	Presence of prey	<i>Oithona davisae</i>
Uchima & Murano (1988)	Mating	<i>Metridia pacifica</i>
Uchima & Hirano (1988)	Prey densities	<i>Euchaeta rimana</i>
Wong (1988)	Presence of prey	<i>Diaptomus minutus</i>
Yen (1988)	Predator-prey and male-female interactions	<i>Centropages hamatus</i>
Ramcharan & Sprules (1989)	Presence of conspecific	<i>Centropages hamatus</i>
Costello et al. (1990)	Turbulence	<i>Temora longicornis</i> , <i>Pseudocalanus elongatus</i> ,
Marrasé et al. (1990)	Turbulence	
Tiselius & Jonsson (1990)	Foraging behaviour	

Dodson & Ramcharan (1991)	Size-specific behaviour	<i>Paracalanus parvus</i> , <i>Centropages typicus</i> ,
Van Leeuwen & Maly (1991)	Mating	<i>Centropages hamatus</i> , <i>Acartia clausi</i>
Coughlin <i>et al.</i> (1992)	Swimming and search behaviour	<i>Daphnia pulex</i>
	Turbulence	<i>Diaptomus leptopus</i>
Salz & Alcaraz (1992)	Food environment	<i>Amphiprion perideraion</i> larvae
Tiselius (1992)	Predator flow field	<i>Acartia tonsa</i>
Yen & Fields (1992)	Prey densities	<i>Acartia tonsa</i>
Gallego (1994)	Turbulence	<i>Acartia hudsonica</i>
Hwang <i>et al.</i> (1994)	Food concentration and turbulence	Herring larvae
Saiz (1994)	Infochemicals	<i>Centropages hamatus</i>
Dodson <i>et al.</i> (1995)	Prey quality	<i>Acartia tonsa</i>
Saiz & Kjørboe (1995)	Swimming behaviour	<i>Daphnia pulex</i>
Hwang & Turner (1995)		<i>Acartia tonsa</i>
		<i>Macrosetella gracilis</i> , <i>Oithona nana</i> , <i>Oithona similis</i> ,
		<i>Oncaea venusta</i> , <i>Temora turbinata</i> , <i>Undinula vulgaris</i>
		var. <i>taiwanicus</i>
Kjørboe <i>et al.</i> (1996)	Prey quality	<i>Acartia tonsa</i>
Larsson & Kleiven (1996)	Food concentration	<i>Daphnia magna</i>
Dodson <i>et al.</i> (1997)	Food, light and container	<i>Daphnia hyalina</i> , <i>Daphnia pulicaria</i> , <i>Daphnia magna</i> ,
		<i>Daphnia pulex</i>
Fields & Yen (1997)	Mechanical disturbance	<i>Euchaeta rimana</i> , <i>Oithona</i> , <i>Labidocera madurae</i> ,
		<i>Pleuromamma xiphias</i> , <i>Acartia tonsa</i>
Bundy <i>et al.</i> (1998)	Prey perception	<i>Diaptomus sicilis</i>
Brewer (1998)	Mating	<i>Daphnia pulicaria</i>
Caparroy <i>et al.</i> (1998)	Turbulence	<i>Centropages typicus</i>
Doall <i>et al.</i> (1998)	Mating	<i>Temora longicornis</i>
Strickler (1998)	Mating	<i>Cyclops scutiger</i>
Tsuda & Miller (1998)	Mating	<i>Calanus marshallae</i>

Weissburg et al. (1998)	Mating	<i>Temora longicornis</i>
Yen et al. (1998)	Mating	<i>Temora longicornis</i>
Baillieux & Blust (1999)	Infochemicals	<i>Daphnia magna</i>
Kjørboe et al. (1999)	Turbulence	<i>Acartia tonsa</i>
Mazzocchi & Paffenhöfer (1999)	Swimming behaviour	<i>Clausocalanus furcatus</i>
Broglio et al. (2001)	Prey quality	<i>Acartia clausi</i>
Schmitt & Seuront (2001)	Swimming behaviour	<i>Temora longicornis</i>
Titelman (2001)	Predator-prey interactions	<i>Acartia tonsa</i> , <i>Temora longicornis</i> , <i>Centropages typicus</i>
Buskey et al. (2002)	Hydrodynamical disturbance	<i>Acartia tonsa</i> , <i>Acartia lilljeborgii</i>
Doall et al. (2002)	Predator-prey interactions	<i>Euchaeta rimana</i> , <i>Acartia fossae</i> , <i>Acrocalanus inermis</i>
Fields & Yen (2002)	Mechanical disturbance	<i>Euchaeta rimana</i>
Leising & Franks (2002)	Presence of prey	<i>Acartia clausi</i>
Paffenhöfer & Mazzocchi (2002)	Swimming behaviour	<i>Oithona plumifera</i>
Weber & Van Noordwijk (2002)	Predator infochemicals	<i>Daphnia galeata</i>
Green et al. (2003)	Predator flow field	<i>Acartia tonsa</i> , <i>Temora longicornis</i> , <i>Eurytemora affinis</i>
Malkiel et al. (2003)	Flow field	<i>Diaptomus minutus</i>
Salz et al. (2003)	Turbulence	<i>Oithona davisae</i>
Titelman & Kjørboe (2003a)	Stage and species specific variability	<i>Centropages typicus</i> , <i>Calanus helgolandicus</i> , <i>Temora longicornis</i> , <i>Acartia tonsa</i> , <i>Eurytemora affinis</i> , <i>Euterpina acutifrons</i>
Titelman & Kjørboe (2003b)	Predator avoidance	<i>Centropages typicus</i> , <i>Calanus helgolandicus</i> , <i>Temora longicornis</i> , <i>Acartia tonsa</i> , <i>Eurytemora affinis</i> , <i>Euterpina acutifrons</i>
Nihongi et al. (2004)	Mate-searching	<i>Leptodiaptomus ashlandi</i>
Seuront et al. (2004b)	Individual variability	<i>Oncaea venusta</i>
Seuront et al. (2004c)	Swimming behaviour	<i>Daphnia pulex</i> , <i>Temora longicornis</i>
Seuront et al. (2004d)	Turbulence	<i>Daphnia pulex</i> , <i>Temora longicornis</i>
Uttieri et al. (2004)	Light	<i>Daphnia pulex</i>
Yen et al. (2004)	Mating	<i>Temora longicornis</i>

Bagøien & Kiørboe (2005a)	Mating	Centropages typicus
Bagøien & Kiørboe (2005b)	Mating	Acartia tonsa
Kiørboe & Bagøien (2005)	Mating	Centropages typicus, Pseudocalanus elongatus, Acartia tonsa
Kiørboe et al. (2005)	Mating	Pseudocalanus elongatus
Uttieri et al. (2005)	Light	Daphnia pulex

1997), turning angles (e.g., Coughlin *et al.*, 1992) and estimates of track tortuosity (e.g., Buskey, 1984). Despite their utility, these instruments do not take into account all the aspects of motion, such as the Lagrangian and fractal ones, and must therefore be complemented with other parameters.

Part of this thesis work has focused on the development of numerical techniques by which to characterise the two-dimensional swimming tracks of *C. furcatus* in terms of their Lagrangian and fractal properties. Such description will highlight peculiar aspects of the swimming motion of this small copepod, providing useful details for a proper comprehension of its behaviour.

2.2 Quantifying the motion of *C. furcatus*

One of the principal aims supporting this thesis work is the development of new and alternative techniques by which to highlight peculiar aspects of the tracks described in varying experimental conditions.

To achieve this, two different lines of research have been followed. The trajectories of freely swimming *C. furcatus* adult females have been analysed in terms of their Lagrangian and fractal properties, the theory and methodologies of which will be discussed in the following sections. These approaches have been utilised to characterise the tracks described in presence of prey item, either capturing them or not. This allows investigation of whether the presence of a quarry determines a change in the paths and in their properties.

2.2.1 Data set

The numerical analysis has been applied to swimming trajectories of *C. furcatus* recorded by Mazzocchi at the Skidaway Institute of Oceanography. Detail of methods utilised in copepod recording can be found in Mazzocchi & Paffenhöfer (1999). In brief, copepods had been filmed using a modified CritterCam® (Strickler, 1985), with a

sampling frequency set to 60 fields s^{-1} . The field of view was 16×10 mm, with a resolution of $\sim 15 \mu m$. Adult *C. furcatus* females were placed in 3 l vessels filled with copepod preconditioned filtered sea water. The vessel was covered with a glass plate to avoid any wind-induced turbulence. Copepods were observed in dark conditions, at a temperature of 20°C. During the experiments, the females were offered a mixed diet of the dinoflagellate *Gymnodinium nelsoni* and of the flagellate *Rhodomonas* sp.. Due to their size and to the resolution of the video equipment, only dinoflagellates were clearly recognisable in the filming. A Panasonic AG-7355 video cassette recorder was connected to a Sony Triniton monitor and to a PC equipped with an IMAQ PCI-1408 board. The synchronization between the video cassette recorder and the PC was ensured by a For.A FA-310P digital time-base corrector. Selected sequences have been manually digitised using a code implemented by "e-voluzione" (s.r.l., Napoli), by which the position of the head of the copepod, taken as a reference point, could be tracked for the entire time length of the clip. As in Doall *et al.* (1998), the rostrum of the copepod was the reference point tracked for each trajectory.

As a selective criterion, the recorded tapes (for a total of approximately 6 h of recordings) have been analysed to select all the clips in which *C. furcatus* was in focus for at least 2s. This choice allows analysing the motion for a time span sufficient enough to calculate statistically significant measures and characteristics. The numerical analysis was applied to a total of 92 sequences, described by all the 8 different specimens recorded at different times of the day. The sequences were divided into two classes: the first (CPT: 36 sequences) collecting all the clips in which the trajectory was associated with the capture of a prey; the second (SWM: 56 clips) in which were grouped the sequences showing the copepod swimming very close to a prey, but without trying to catch it.

Data were preliminarily checked for the presence of spikes due to digitalisation errors. The data utilised for the numerical analyses were not smoothed, to avoid losing the small-scale information associated with raw data.

2.2.2 Lagrangian approach

The study of the motion of a fluid can be generally approached by two different perspectives, namely the Eulerian and the Lagrangian ones. In the former, the variables are measured at fixed locations (Pickard & Emery, 1982; Yamazaki & Okubo, 1995), thus being only a function of time. For that reason, Eulerian description is often referred to as a “fixed-point” analysis since the observer is “fixed” in the space (Williams & Elder, 1989; Legendre & Legendre, 1998).

Lagrangian analysis, on the other hand, “follows” the particles which are dispersed in the analyzed fluid (Pickard & Emery, 1982; Yamazaki, 1993; Yamazaki & Okubo, 1995), and the observer “moves” with the fluid (Williams & Elder, 1989; Legendre & Legendre, 1998). In other words, each particle is followed in space and time (Emery & Thomson, 1998) and its trajectory is reconstructed and characterized.

Lagrangian analysis has long been used in physical oceanography to describe the transport of drifting buoys (Pickard & Emery, 1982; Zambianchi & Griffa, 1994; Özgökmen *et al.*, 2000). Being related to trajectories, the Lagrangian approach seems to fit perfectly with the need to characterize the paths of zooplanktonic organisms. This *modus operandi* allows the evaluation of the strictly kinematic properties of the routes of a generic tracer, properties that cannot be otherwise determined. By kinematics it is intended the analysis of the motion without regard to the forces that generated it (Beatty, 1986). In the framework of zooplankton swimming routes, this approach is rather innovative. A preliminary application of this procedure to zooplankton tracks is provided by Uttieri *et al.* (2004), who characterised the three-dimensional trajectories described by the freshwater cladoceran *Daphnia pulex* swimming in two different light conditions. The results obtained by Uttieri *et al.* (2004) demonstrate the reliability of this approach, by which it is possible to classify and compare trajectories effectively, thus improving our knowledge about the intimate behaviour of zooplanktonic organisms. For the first time, in this thesis these techniques are utilised for the characterization of the motion of a marine copepod.

As in Uttieri *et al.* (2004), the swimming motion of adult females *C. furcatus* is characterised in terms of three Lagrangian tools, namely:

- 1- velocity autocovariance (Kundu, 1990);
- 2- spectral analysis (Press *et al.*, 1989; Kundu, 1990; Emery and Thomson, 1998);
- 3- reconstruction of the kinetic energies associated to the motion (Patterson, 1985).

2.2.2.1 Velocity autocovariance

The velocity autocovariance gives an estimate of the randomness of the motion by estimating for how long the velocity at a generic time step t will influence later motion. In the present work, this parameter has been calculated for the two velocity components (u and v respectively along X and Y) of the swimming trajectories of *C. furcatus*.

Velocities are computed using a central difference method. For instance, along the X direction the velocity at a certain point of the trajectory (x_i) is calculated as:

$$u(x_i) = \frac{x_{i+1} - x_{i-1}}{2\Delta t}$$

where Δt is the time lag between two consecutive points.

The autocorrelation of the velocity at a generic time t with the velocity itself at a later time $t+\tau$ can be expressed as (Bendat & Piersol, 1966):

$$R(\tau) = \overline{u(t) u(t + \tau)}$$

the overbar representing an average over the entire time length considered. The normalized autocorrelation function or, simply, the autocovariance (Kundu, 1990), is obtained by dividing the previous equation by $\overline{u^2}$ (mean square velocity):

$$r(\tau) = \frac{\overline{u(t) u(t + \tau)}}{\overline{u^2}}$$

By definition, the autocovariance is a measure of the “covariance of the series with itself” (Legendre & Legendre, 1998); alternatively, we can think of $r(\tau)$ as an estimate of the degree of correlation of the velocity with itself at subsequent times.

For short memory velocities we expect $r(\tau)=1$ just for $\tau=0$, i.e. velocity is totally correlated only with its own instantaneous value. Such randomness implies little self-correlation at distant time, therefore, for $\tau>0$, $r(\tau)$ will decrease and asymptotically approach zero. A velocity showing long-term memory, vice versa, would have persistent values of the autocovariance over all time displacements.

The “memory” of the process is represented by the parameter T , the integral time scale, given by (Kundu, 1990):

$$T = \int_0^{\infty} r(\tau) d\tau$$

which is an estimate of how long the velocity at a certain time influences the later motion. As T is the area under the curve of the autocorrelation function, the faster the curve approaches zero, the smaller the area, and consequently the shorter the process memory; this parameter has long been used in physical oceanography (e.g., Griffa, 1996) for the characterization of drifting buoys tracks. In the following, the notation T_x and T_y will be utilised to distinguish between the integral time scales associated respectively to the velocity components u and v .

2.2.2.2 Spectral analysis

This technique allows a description of the peak frequencies (if any) associated with the swimming motion. A time series of data (such as, displacements of velocities) can be regarded as a function of time or of frequency. These two descriptions are correlated, and it is possible to switch from one to the other by means of the Fourier transform (Press *et al.*, 1989; Emery & Thomson, 1998; Legendre & Legendre, 1998). A trajectory can be considered as a stochastic time series of organism positions; therefore, a typical tool conceived for the analysis of time series, the power spectral density function $S(\omega)$ (PSD in the following), can be adopted:

$$S(\omega) = \frac{1}{2\pi} \int_{-\infty}^{\infty} e^{-i\omega\tau} R(\tau) d\tau$$

describing the data in terms of their peak frequency composition (Emery & Thomson, 1998). As evident from the equation, the PSD is the Fourier transform of the autocorrelation function $R(\tau)$; as a consequence, the results obtained by these two applications are complementary and give a more exhaustive description of the analyzed phenomenon.

In the present work, the PSD has been determined by applying the fast Fourier transform (FFT) (Press *et al.*, 1989; Legendre & Legendre, 1998) directly to the data series (in this case, velocity). This procedure allows a faster determination (in terms of computational time) of the spectrum without any loss of information.

The PSD function represents the way the energy associated to the process is distributed over the spectrum of frequencies (Kundu, 1990; Emery & Thomson, 1998; Legendre & Legendre, 1998). The presence of peaks in the PSD corresponds to dominant frequencies in the motion; conversely, the absence of a defined slope in the graph is representative of no significant velocity correlations. In the latter case, the spectrum of a zero memory process is defined as "white", as it is represented by a fairly constant value over the whole frequency range (Bendat & Piersol, 1966; Emery & Thomson, 1998).

2.2.2.3 Reconstruction of kinetic energies

Based on the kinematic or Reynolds decomposition, the velocity \underline{u} of a parcel of fluid or of a body moving in it can be considered as the sum of a mean field ($\overline{\underline{u}}$) and of a superimposed stochastic one (\underline{u}') (Pond & Pickard, 1983; Williams & Elder, 1989; Mann & Lazier, 1996):

$$\underline{u} = \overline{\underline{u}} + \underline{u}'$$

where the underbar is indicative of vectorial notation.

The reconstruction of the kinetic energies associated with the different components of the motion allows the determination of the relative importance of the random

component of the movement over the total energy budget of the motion itself, as well as the characterization of the strictly kinematic properties of the motion.

Following the kinematic decomposition, it is possible to define three distinct kinetic energies. The total kinetic energy (TKE) per unit mass is:

$$TKE = \frac{\bar{u}^2}{2}$$

whereas the kinetic energies associated with the mean (MKE) and stochastic (eddy) (EKE) field are, respectively:

$$MKE = \frac{\bar{u}^2}{2}$$

$$EKE = \frac{\overline{u'^2}}{2}$$

It is worth noticing that the above energies are kinematic properties associated with the organisms' motion, and do not reflect any metabolic process.

In this work, attention has been concentrated to the $\frac{EKE}{TKE}$ ratio over the XY plane, by combining the two velocity components (Patterson, 1985). For the sake of clarity, the kinetic energies associated with the XY plane are given by:

$$TKE = \frac{\bar{u}^2 + \bar{v}^2}{2}$$

$$MKE = \frac{\bar{u}^2 + \bar{v}^2}{2}$$

$$EKE = \frac{\overline{u'^2} + \overline{v'^2}}{2}$$

The $\frac{EKE}{TKE}$ ratio determines the relative importance of the random component of the velocity in the overall motion, and is therefore an estimate of the irregularity of the motion itself; values close to 1 are typical of random processes, for which most of the total kinetic budget is due to the stochastic part.

The mean flow that, through the kinematic decomposition, needs to be evaluated in order to derive the random part is the result of averaging the velocities in sub

regions ("bins") of the domain spanned by the organisms. The spatial domain has been divided into square bins in each of which a mean velocity is calculated; then, per each point inside that bin, the random component of the velocity is computed using the Reynolds decomposition.

Attention has been paid to the dimension of the bins (b_s) used (e.g., Poulain & Niiler, 1989); small bins, in fact, take into account the small-scale variability of the process, whereas large-sided ones contain more points in them and so the averaging of the velocities is more reliable (Patterson, 1985). Moreover a crucial point for the operational usefulness of the kinematic decomposition is the presence of a scale separation between the mean and the stochastic portions, i.e. b_s has to be much larger than the typical length scale of the displacement fluctuations. For these reasons, per each trajectory the evaluation of the different E_{KE}/T_{KE} ratios has been reiterated over a range of b_s ; not only do these values respect the scale-separation, but also they are large enough so that a minimum of 15 points is present in every bin, thus making the averaging statistically consistent.

2.2.3 Fractal analysis

2.2.3.1 An overview on fractal geometry

According to the classic Euclidean geometry, objects can be characterised by means of their dimensions. Namely, a point has an Euclidean dimension (E) equal to 0, a line has $E=1$, whereas for a surface and a solid the Euclidean dimensions are respectively 2 and 3. Unfortunately, real objects have shapes that hardly resemble geometrical items: they are irregular, complex, fragmented. In this case, what is their dimension? Euclidean geometry "idealizes form" (Jelinek *et al.*, 1999), being

inadequate to characterize such kind of items; consequently, a different approach has to be used, namely the fractal geometry.

Mandelbrot (1967) defined natural objects as “fractals” (from the Latin *fractus*, “irregular”), and described their complexity in terms of a new approach, the fractal dimension, measuring the tendency of an object to fill the Euclidean space in which it is defined. The fractal dimension D of an object defines its degree of irregularity: unlike Euclidean (and topological) dimension, D can assume both integer and irrational values (Mandelbrot, 1983). For example, the fractal dimension of a plane curve ranges from 1 to 2: as it approaches one of the limits, the shape will resemble a straight line or a surface correspondingly. Of course, for a straight line: $E=D=1$.

For an object to be regarded as fractal, one fundamental property has to be respected, the self-similarity (or scale invariance) (Mandelbrot, 1983), i.e. the shape of the item has to be retained by the parts composing it. In this way, it is always possible to look at the same morphology (the motif) independently on the scale considered, with a cascade of structurally similar details (Avnir *et al.*, 1998).

The classical and most well known application of the concept explained so far is the determination of the length of a coast, carried out by the English meteorologist Lewis Fry Richardson (Mandelbrot, 1967; Mandelbrot, 1983; Lauwerier, 1991). A coast is a very good example of natural fractal item: it is made of meanders and bays, with a pattern held constant over a wide range of scales. Richardson calculated the length of a coast using the compass method, i.e. covering the shoreline with N tangent circles of radius η ; obviously the smaller η , the greater N . The length $L(\eta)$ of the coast is:

$$L(\eta) \propto \eta^{-\alpha}$$

α was supposed to be a generic exponent. Fractal geometry gave it new emphasis; α could be rewritten as (Mandelbrot, 1983):

$$\alpha = D - 1$$

where D is the fractal dimension.

Fractal dimension can be calculated as (Mandelbrot, 1983):

$$D = \frac{\log(N)}{\log\left(\frac{1}{\eta}\right)}$$

which is a generalized expression used to evaluate the fractal dimension of any fractal object.

Through the years, fractal geometry has gained broad consensus in different fields of application, thus revealing its ability to give useful information about a large variety of phenomena. It is worth noticing that fractal geometry has also been fruitfully applied in non-scientific fields, particularly in computer graphics where its principles have been largely exploited, giving birth to a new form of "art" (Batty, 1985; Jürgens, 1990).

2.2.3.2 Fractals and trajectories

True fractals, characterized by self-similar structures at all scales, can be called "mathematical fractals" (Isliker & Vlahos, 2003), since they are just "beautiful mathematical constructs" (Avnir *et al.*, 1998) or "abstractions" (Bradbury *et al.*, 1984); in such cases D can be easily calculated (Buczowski *et al.*, 1998). Natural objects, on the other hand, can be considered "finite fractals" (Isliker and Vlahos, 2003) in that self-similarity is maintained only over a restricted range of scales (Bradbury *et al.*, 1984; Jelinek *et al.*, 1999); moreover, very often they do not display exactly the same motif at different scales (Jelinek *et al.*, 1999). For this reason, dealing with real items it is convenient to consider scale invariance in a statistical sense (Lauwerier, 1991; Hutchinson, 1995; Jelinek *et al.*, 1999), i.e. each part of the item share the same statistical features of form (Mark, 1984; Lauwerier, 1991; Hutchinson, 1995; Jelinek *et al.*, 1999). The fundamental pre-requisite for fractal characterisation is that self-similarity has to be respected over as many orders of magnitude as possible (Avnir *et al.*, 1998); in addition, unlike true fractals, the fractal dimension of physical objects has to be estimated rather than calculated (Buczowski *et al.*, 1998).

One classical case of statistical scale invariance is the Brownian motion (Mandelbrot, 1983; Lauwerier, 1991; Hutchinson, 1995), which is also an example of a random walk, a succession of displacements each independent on the other (Csanady, 1973). On this ground, random walks can be analysed by means of fractal dimension obtaining precious information about their spatial complexity. Random walks can be strongly convoluted and, to an extent, they tend to fill all the space around them, thus gaining D values equal to 2 (Mandelbrot, 1983; Torrens, 2002). The applications are however not restricted just to random paths, fractal dimension having indeed been successfully applied to different kinds of trajectories, including zooplankton (Table II). Recently, new methodologies for the analysis of three-dimensional paths have also been evolved (Seuront *et al.*, 2004a; Uttieri *et al.*, 2005).

Table II: application of fractal geometry to different kinds of trajectories, arranged in chronological order.

Author	Typology of Trajectory
Dicke & Burrough (1988)	Mite trails
Sanderson & Goulding (1990)	Lagrangian drifters
Coughlin <i>et al.</i> (1992)	Zooplankton swimming
Johnson <i>et al.</i> (1992)	Tenebrionid beetle
Meyers <i>et al.</i> (1994)	Numerical model
Mullen & Kirwan (1994)	Lagrangian drifters
Nakamoto <i>et al.</i> (1994)	Lagrangian drifters
Erlandsson & Kostylev (1995)	Marine prosobranch
Provenzale <i>et al.</i> (1995)	Numerical model
Wiens <i>et al.</i> (1995)	Insects
Bascompte & Vilà (1997)	Wolf
Claussen <i>et al.</i> (1997)	Turtles
Hansen <i>et al.</i> (1997)	Capillary waves
Westcott & Graham (2000)	Birds
Schmitt & Seuront (2001)	Zooplankton swimming
Doerr & Doerr (2004 and 2005)	Birds
Nams (2005)	Numerical model, deer mouse and red-backed vole
Seuront <i>et al.</i> (2004a, b, d)	Zooplankton swimming
Uttieri <i>et al.</i> (2005)	Zooplankton swimming

The major point in using fractal geometry for the characterization of trajectories lies in its scale-independency, as recently discussed in Seuront *et al.* (2004a and b).

Buskey (1984) developed the net-to-gross displacement ratio as an index of track tortuosity, but this parameter is influenced by the physical and temporal scales adopted. Fractal dimension, on the other hand, is free from these scale-dependencies, and offers a more precise numerical characterization of the degree of involvedness of a path (Seuront *et al.*, 2004a and b; Uttieri *et al.*, 2005).

2.2.3.3 The box-counting method

Different techniques have been evolved for the evaluation of fractal dimension, among which the compass method (Mandelbrot, 1983; Coughlin *et al.*, 1992), the yardstick method (Sanderson & Goulding, 1990; Mullen & Kirwan, 1994; Hansen *et al.*, 1997), the cumulative mass method (Caserta *et al.*, 1995), the extended counting method (Sandau, 1996; Sandau & Kurz, 1996) and cluster analysis (Lana *et al.*, 2005)

The box counting method (henceforth BCM) is probably the most popular, and has been widely used in scientific applications. The Euclidean space containing the fractal item is divided into sub-regions by superimposing a grid of non-overlapping square elements of side length r ; then, the number $N(r)$ of boxes containing the fractal is counted. Following Ott (1993), all boxes are equally “important”, i.e. all boxes containing at least one point of the item are taken into consideration. This box-counting is repeated over a range of r -values, and in the end the $\log(N(r))$ is plotted as a function of $\log(r)$. The slope of the least-squares regression line, taken with a minus sign, represents the fractal dimension D of the fractal item (Sreenivasan *et al.*, 1989; Soddell & Seviour, 1995; Sandau & Kurz, 1996; Wijesekera, 1996). D values depend just on the number of boxes occupied, and not on the total number of boxes covering the Euclidean space, demonstrating the reliability of this estimation which is not influenced by the extension of the reference frame.

The adoption of the BCM presents two critical steps, which need specific care in order to avoid misinterpretations and wrong estimates. These points have been recently discussed in Uttieri *et al.* (2005), and are represented by the choice of the r -values and by the positioning of the grid.

In this work, D values of *C. furcatus* swimming trajectories have been estimated using a modification of the methodology proposed by Uttieri *et al.* (2005), who developed a technique by which to evaluate D values for three-dimensional trajectories, using a code implemented in MATLAB (MathWork, Inc.). As a preliminary step, the presence of an intermittent pattern in the displacements has been verified, thus ensuring the possibility of considering the trajectories as statistically self-similar. A virtual square element grid of side length r is superimposed on the Euclidean spatial domain containing the trajectory; each point of the track is then associated with one cube of the lattice by arranging its coordinates using a procedure similar to that developed by Liebovitch & Toth (1989). Following Uttieri *et al.* (2005), the range of r values used in D estimation consists of a 2^n sequence, and the lattice is placed with its lower and left margins tangential to the trajectory (as suggested by Buczkowski *et al.*, 1998). As suggested in Uttieri *et al.* (2005), a calibration of the technique has also been performed, with the aim of retrieving a correction factor necessary for the correct estimation of D values.

2.2.4 Statistical analysis

The results obtained using the methodologies described above have been analysed using a one-way analysis of variance (ANOVA) (Sokal & Rohlf, 1995) to evaluate if any significant difference in the tracks described in association with a capture or not was evident.

To give a graphic representation of the statistical analysis, the ANOVA has been complemented with the boxplots method (Tukey, 1977; McGill *et al.*, 1978; Sokal & Rohlf, 1995). For each sample (i.e., one class of motion), a box and whisker plot is drawn: the bottom and the top of the box are respectively the 25th and 75th quartiles of the range, whereas the line inside it is the median. The whiskers extending from the two ends of the box show the extent of the rest of the data; values beyond the end of the whiskers are represented with a cross; if no data are present beyond the box, a

dot is drawn at the bottom of the whisker. The length of the box is the interquartile range, also referred to as dispersion (in a statistical sense). Two samples can be considered statistically similar when the median lines overlap each other and the dispersions are comparable.

The degree of fitting of the least-squares regression lines for D evaluation has been evaluated using the coefficient of determination r^2 (Sokal & Rohlf, 1995), which is a normalised index indicating how reliable D estimates are.

2.3 The swimming trajectories of *C. furcatus*

2.3.1 Lagrangian characterization

Figure 2.1 reports some examples of swimming trajectories described by freely swimming specimens of *C. furcatus*.

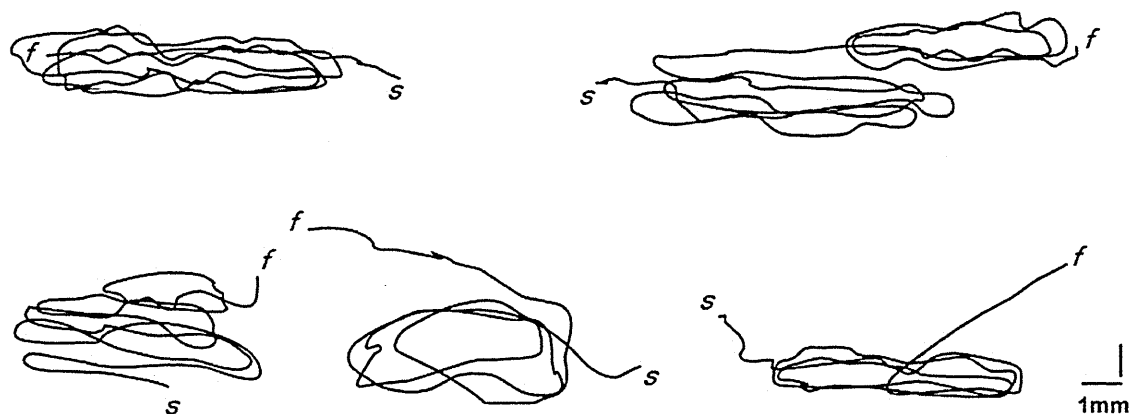


Figure 2.1: examples of swimming trajectories described by freely swimming adult females of *C. furcatus*. The paths were obtained from digitised tracks, splined using Matlab and subsequently smoothed, for graphical reasons, using Paint Shop Pro. Label (s and f) respectively indicating starting and ending point. All tracks were described in 8 seconds.

In both classes of motion (i.e., those involving the capture of a prey item, CPT, and those not associated with a feeding event, SWM), the autocovariance of the two velocity components (u and v) rapidly drops to zero after a few time steps (Figure 2.2), with a delta shaped pattern reminiscent of a random walk. This indicates that the velocities are characterised by short autocorrelation, and that the associated T values are small (SWM: $T_x=0.68 \pm 0.32$ s, $T_y=0.75 \pm 0.38$ s - CPT: $T_x=0.71 \pm 0.27$ s, $T_y=0.70 \pm 0.25$; mean value \pm standard deviation). The results obtained for the two sets of trajectories are statistically similar ($p=0.2918$; boxplots in Figure 2.3), indicating that the capture of prey items does not determine any change in the Lagrangian characteristics of the swimming tracks.

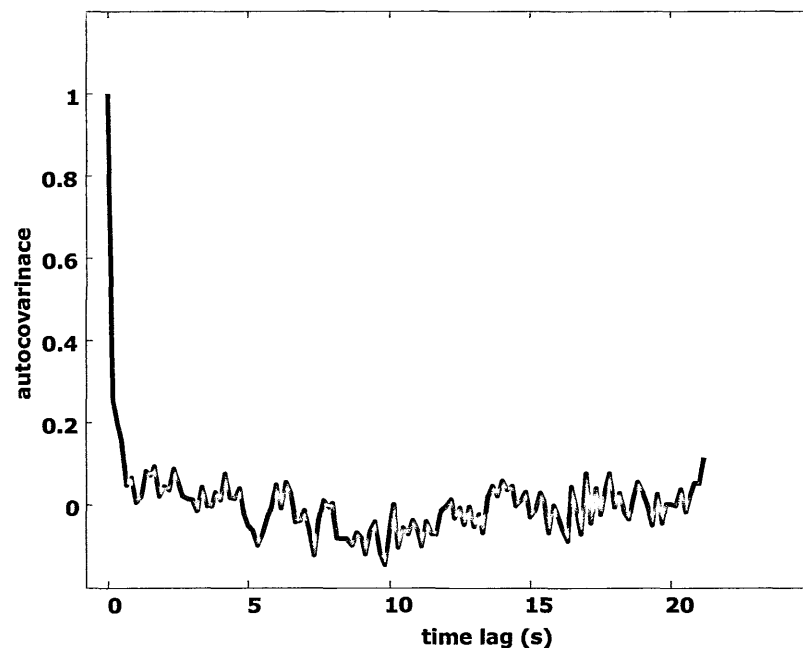


Figure 2.2: autocovariance of one velocity component of a freely swimming *C. furcatus*; the curve rapidly approaches zero, with a delta-shaped pattern typical of uncorrelated random walks.

The characterisation by means of the spectral analysis highlights that the motion can be assumed as the superimposition of a large number of frequencies. In fact, no

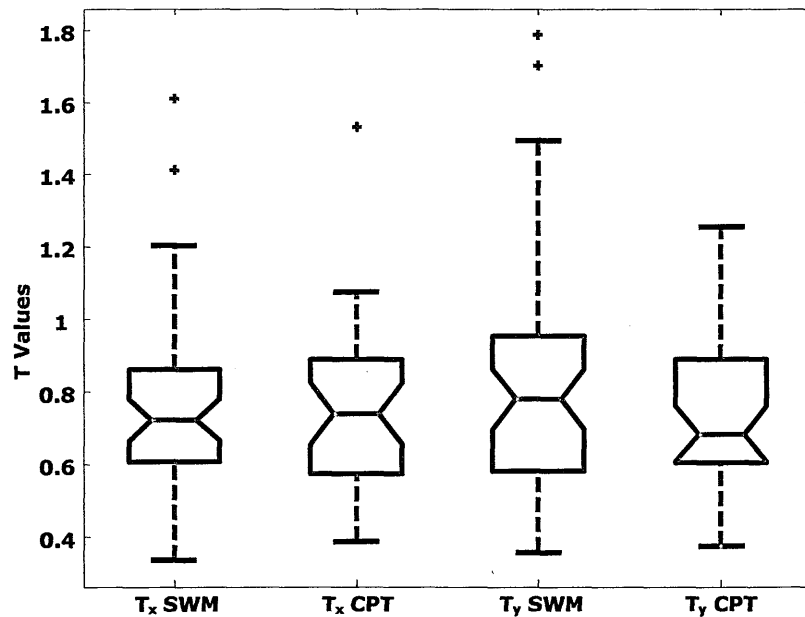


Figure 2.3: boxplots of the integral time scale T evaluated for the two velocity components of the trajectories described in each class of motion.

dominant peak is recognisable in the PSD plots (Figure 2.4), with a white spectrum typical of processes lacking a relevant memory.

The evaluation of the kinetic energies reveals that, in both CPT and SWM, there is a relevant random component in the paths, in accordance with that previously noticed with the autocovariance and the spectral analysis. More particularly, the role of EKE over the total kinetic budget of the track is evident from EKE/TKE values (SWM: 0.97 ± 0.01 - CPT: 0.97 ± 0.02 ; mean value \pm standard deviation), which indicates that more than 95% of the energy is due to the random component of the motion. Also in this case, results are statistically similar ($p=0.1878$; boxplots in Figure 2.5), no difference occurring between the two classes of motion.

Details on the values attained by each track analysed are reported in Appendix I.

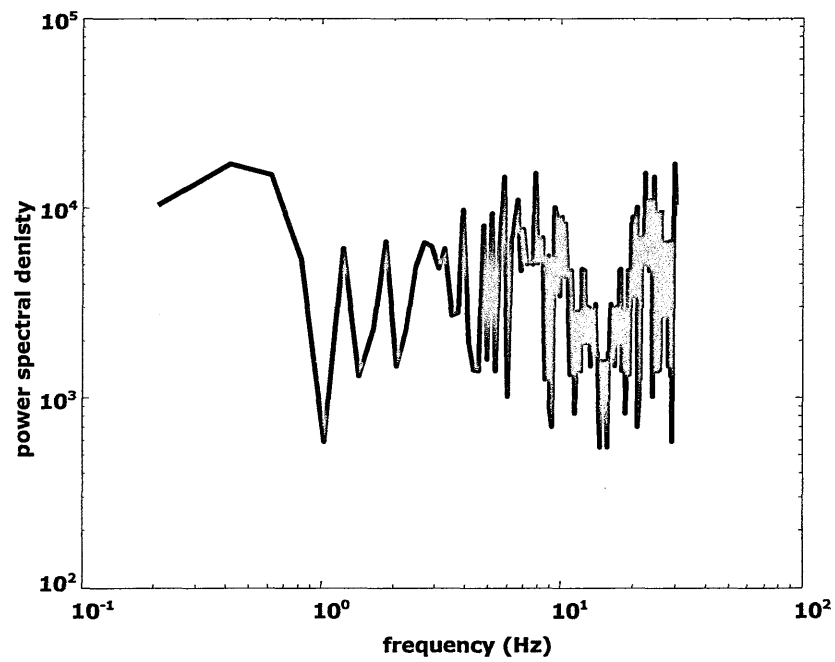


Figure 2.4: power spectral density plot calculated on one velocity component of a *C. furcatus* swimming trajectory.

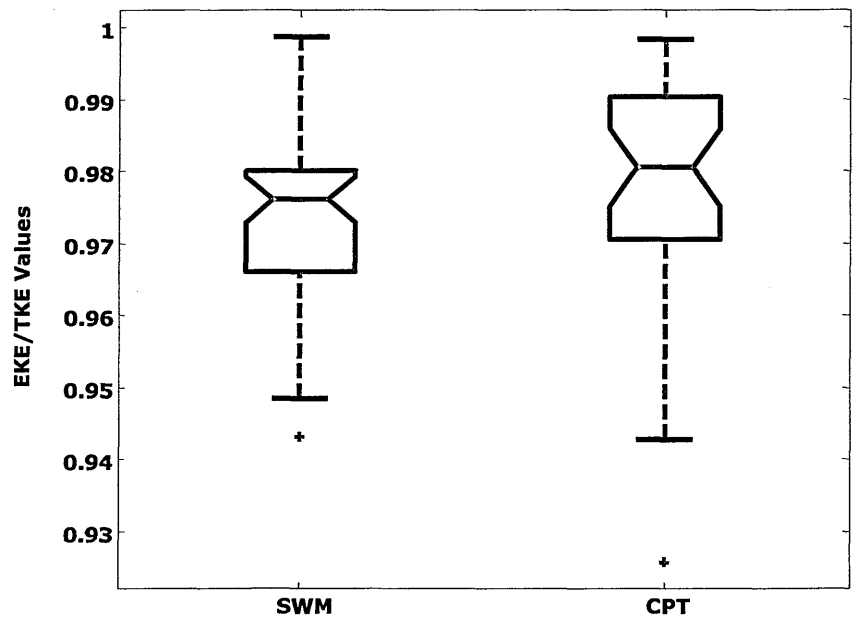


Figure 2.5: boxplots of the $\frac{EKE}{TKE}$ values for the two classes of motion considered (SWM and CPT).

2.3.2 Fractal characterization

The trajectories of *C. furcatus* present an intermittent pattern in the displacements (Figure 2.6), indicative of a fractal structure. The tracks show low fractal dimension values (SWM: $D=1.10 \pm 0.08$ - CPT: $D=1.14 \pm 0.08$; mean value \pm standard deviation), independently of the class of motion analysed. No significant difference occurs between the different classes analysed ($p=0.2463$; boxplots in Figure 2.7). The regression lines of the log-log plot always fit the points with good accuracy (SWM: $r^2=0.96 \pm 0.01$ - CPT: $r^2=0.97 \pm 0.01$; mean value \pm standard deviation), thus validating the reliability of the results (Figure 2.8).

These results indicate that the swimming tracks of *C. furcatus* spread preferentially along one direction only, and that the presence of capture events does not determine any modification in the complexity of the swimming tracks. Appendix I provides a report of the values of fractal dimension attained by each of the tracks considered in this Chapter.

2.4 Discussion

The characterization of the swimming trajectories described by *C. furcatus* provides useful details on the properties associated with its small-scale motion. Such information provides new data by which to gain a more comprehensive description of the behaviour of the copepod.

As described by Mazzocchi & Paffenhöfer (1999), the swimming motion of *C. furcatus* is characterised by repetitive looping performed at a mean speed of 10 mm s^{-1} , corresponding to 10 bl s^{-1} . The numerical analysis of the video recording performed in this work has highlighted that this typical behaviour is maintained by different females, without showing significant variability among the individuals examined, as reported for other copepods (Turner *et al.*, 1993; Seuront *et al.*, 2004b). In addition,

the characteristic swimming habit is maintained over 24 h and is displayed by egg-carrying females as well, supporting its species-specificity aspects.

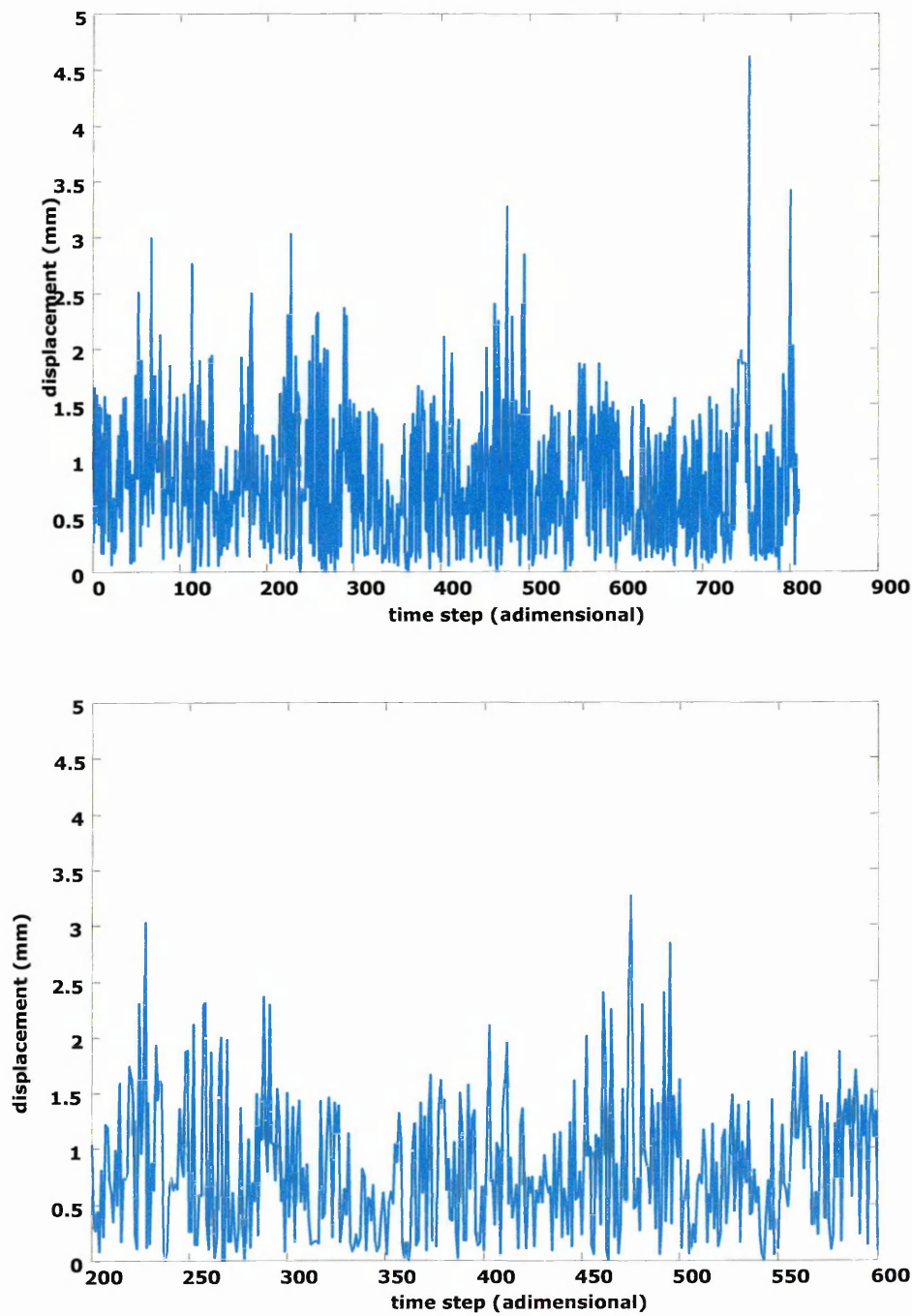


Figure 2.6: intermittent structure of the two-dimensional displacements of *C. furcatus*. The large (above) and small (below) scale patterns are similar, indicating self-similarity over different scales.

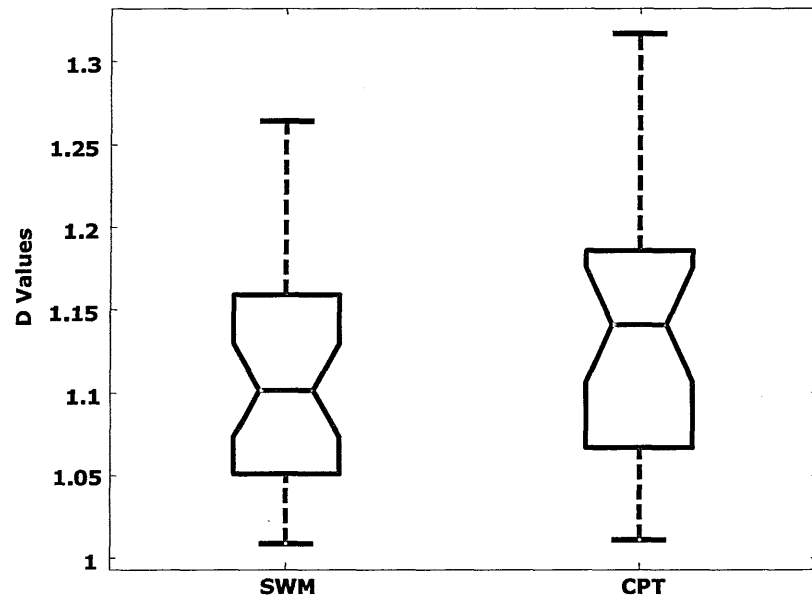


Figure 2.7: boxplots of the D values for the swimming trajectories associated (CPT) and not associated (SWM) to a feeding event.

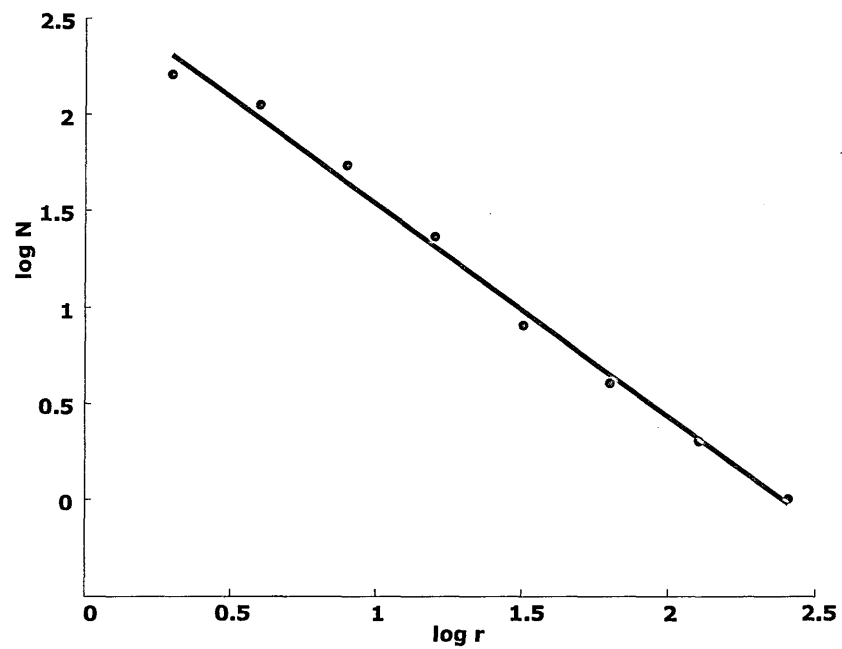


Figure 2.8: least-squares regression line for one trajectory described by a freely swimming *C. furcatus* ($r^2=0.99$).

For the first time, the swimming trajectories described by a freely swimming marine copepod have been characterised in terms of their kinematic properties using a Lagrangian approach. In the field of zooplankton behaviour, these techniques have been so far utilised only in the description of the motion of the freshwater cladoceran *Daphnia pulex* (Uttieri *et al.*, 2004). The comparison of the results obtained for *C. furcatus* and for *D. pulex* highlights some interesting common features. The copepod and the cladoceran show completely different swimming behaviour: while the former swims continuously in a highly convoluted mode, the former adopts a much simpler motion, characterised by small hops resulting in simpler and straighter tracks. Despite these differences, the kinematic properties of the motion of the two zooplankters are roughly the same. In both cases, the autocovariance sharply drops to zero after few time steps, with associated small T values; moreover, for both *C. furcatus* and *D. pulex*, the EKE/TKE is close to 1 and the PSD plots lack any sharp peak characterising the motion. It therefore emerges that the swimming motions of two organisms are characterised by the same kinematic properties, which may consequently be utilised as a common strategy to maximise the success in aquatic environments.

Besides their numerical values, the parameters discussed in the previous sections show that the motion of *C. furcatus* has properties similar to those of a random walk, with swimming trajectories showing a relevant random component. Such characteristics are not influenced by the capture of a prey, and can therefore be considered as typical of the swimming activity of the copepod. In addition, the lack in a peak frequency in the motion can be interpreted as a strategy to avoid predation; in this case, *C. furcatus* would not “play possum”, but would rather exploit its behaviour as a “fluid-dynamical camouflage” (Hwang & Strickler, 2001). This may explain the low predatory rates by chaetognaths on *C. furcatus* reported by Kehayias *et al.* (1996): these authors report that, even though *C. furcatus* was the most abundant copepod in the field, the electivity index was negative. They argued that a possible explanation would be represented by different vertical positions of the copepod and of its predator, but the data in Fragopoulou *et al.* (2001) indicate that both predator and

prey occupy the same layers. Hence it is reasonable to hypothesise that the low predatory rates are due to the swimming behaviour of the copepod. Chaetognaths' feeding bouts are triggered by specific frequencies (Feigenbaum & Reeve, 1977); the absence of a dominant peak of motion may elude the predator, which might lack an important cue for the detection of the copepod. The presence of a white spectrum would therefore act as a mechanical self-hiding defence, as already proposed for the freshwater cladoceran *D. pulex* (Uttieri *et al.*, 2004), counteracting the stronger signal created in the medium owing to their continuous motion (as discussed in Paffenhöfer & Knowles, 1980, for copepod nauplii).

The low values of fractal dimension of the trajectories of *C. furcatus* indicate that the tracks have a reduced tendency to occupy all the space available, being rather elongated along one direction. This represents a numerical confirmation of the small-volume exploitation strategy proposed by Mazzocchi & Paffenhöfer (1999), which could probably increase the encounter rate with prey items thus balancing the increased energy expenditure due to the continuous motion. Such behaviour has some resemblance with the area-restricted search foraging strategy (Tinbergen *et al.*, 1967) adopted by different organisms (as reviewed in Leising, 2002) including plankton (e.g., Gallego, 1994; Villanueva *et al.*, 1996; Leising, 2002; Leising & Franks, 2002). Based on this strategy, an animal increases its turning rate when in presence of prey items, describing more tortuous tracks (i.e., tracks with higher D values); as a result, copepods modify their swimming motion when exposed to food (e.g., Buskey, 1984; Wong & Sprules, 1986; Uchima & Hirano, 1988; Wong, 1988; Tiselius, 1990). In *C. furcatus*, instead, the degree of complexity of the trajectories does not vary as a function of the captures on *G. nelsoni*; consequently the swimming motion of the copepod can be assumed as already optimised for the occurrence of prey items.

Owing to the strictly two-dimensionality of the data, it is not possible to gather any indirect information on the three-dimensional fractal characteristics of *C. furcatus* swimming motion. As demonstrated in Uttieri *et al.* (2005), the characterization of two-dimensional projections of a three-dimensionally evolving motion cannot be

utilised to evaluate the degree of complexity of a track described in three dimensions, which must instead be analysed *per se*. However, modelling results by Wiggert *et al.* (2005) show that, in a three-dimensional reference frame, the swimming motion of *C. furcatus* consists of a series of continuous loops in the horizontal plane alternated with straight vertical movements which allow the copepod to rapidly move in different regions of the fluid.

Direct observations show that the typicality in the swimming motion is maintained for up to 7 hours also by specimens kept in filtered sea water. The only difference noted relies in the extension of the loops described by *C. furcatus*: in filtered sea water the loops appear larger, suggesting that the copepod probably scans wider portions of fluid in search for food. This indicates that the natatorial performance is an inborn trait for *C. furcatus*, and it is not modified or affected by the presence of prey items.

The characterisation of the swimming trajectories by means of the proposed analytical tools has highlighted some peculiarities of the motion of *C. furcatus*. The results obtained provide new data for the comprehension of the unique behaviour of this copepod, as well as on its interactions with the surrounding environment. In addition, the methodologies utilised have proved their usefulness in comparing trajectories associated or not with a capture event.

Chapter 3

Prey capture in *Clausocalanus furcatus*

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3.1 Capturing a prey

One of the major goals in copepods' life is capturing and ingesting prey efficiently and in an amount sufficient to cover the daily energetic costs associated to both basic metabolic requirements and swimming activities (e.g., Vlymen, 1970; Klyashtorin & Yarzhombek, 1972; Haury & Weihs, 1976; Klyashtorin, 1978; Klewowski & Sazhina, 1985; Alcaraz & Strickler, 1988; Morris *et al.*, 1990).

Prey capture is profoundly affected by the natatory activity of the predator (as previously discussed in Chapter 2), as well as by the patterns of prey distribution which, in pelagic systems, are variable in both space and time (Boyd, 1996). Swimming behaviour is in turn modified by the alimentary conditions experienced (e.g., prey availability, food quality and/or quantity, etc.) (e.g., Williamson, 1981; Tiselius, 1992; Broglio *et al.*, 2001; Leising & Franks, 2002). Moreover, feeding activity and rates are affected by turbulence (e.g., Costello *et al.*, 1990; Marrasé *et al.*, 1990; Saiz, 1994; Kiørboe & Saiz, 1995; Saiz & Kiørboe, 1995; Visser *et al.*, 2001; Saiz *et al.*, 2003). Feeding success will however also depend on other factors, among which prey behaviour (e.g., Broglio *et al.*, 2001) and escape ability (e.g., Yen & Fields, 1992).

The question of how copepods feed has been debated since Cannon (1928) and Storch (1929). Cannon (1928) noticed the formation of feeding currents created by the beating of the feeding appendages (as later confirmed by high-speed cinematography by Rosenberg, 1980), particles being collected by the "scoop net" filtering of the maxillae (Gauld, 1964).

These observations led Jørgensen (1966) to define copepods as passive filter feeders, retaining particles conveyed to the mouthparts by using them as a sieve. The evidence of food size selection (Richman & Rogers, 1969; Wilson, 1973; Frost, 1977; Landry, 1978 and 1980) and the discovery of numerous chemoreceptors on copepod feeding appendages (Ong, 1969; Friedman & Strickler, 1975; Friedman, 1980) however threw considerable doubt on a purely passive mechanism, and prompted further research on this topic. A breakthrough was represented by the works of

Alcaraz *et al.* (1980) and Koehl & Strickler (1981) who reported that, when a suitable food particle is entrained in the feeding current, a copepod can actively orient and retain it by a "fling and clasp" movement of the second maxillae. These and other observations (e.g., Paffenhöfer *et al.*, 1982; Cowles & Strickler, 1983; Price *et al.*, 1983; Price & Paffenhöfer, 1984 and 1985) sketched a brand-new picture, basing on which copepods could be defined as "suspension feeders" (Paffenhöfer *et al.*, 1982).

Feeding currents are usually displayed by specimens smoothly cruising in the fluid, and their characteristics have been numerically described in detail (e.g., Andrews, 1983; Jiang *et al.*, 1999; Visser & Jonsson, 2000). However, not all cruising copepods feed by means of feeding currents. In some cases, cruising species can prey upon a quarry by performing fast oriented lounges (e.g., Lillielund & Lasker, 1971; Kerfoot, 1978; Tiseluis & Jonsson, 1990; Bundy *et al.*, 1998; Doall *et al.*, 2002), with a mechanism closely recalling that displayed by ambush predators (e.g., *Oithona plumifera*: Paffenhöfer & Mazzocchi, 2002). In these cases, the copepod perceives the presence of a potential prey by detecting stimuli (either mechanical and/or chemical) originating from it. Such cues elicit very fast quarry-oriented responses (i.e. an attack) (Bundy *et al.*, 1998; Svensen & Kiørboe, 2000; Doall *et al.*, 2002; Paffenhöfer & Mazzocchi, 2002). The analysis of such predation events allows the reconstruction of an attack volume (Doall *et al.*, 2002), representing the area of interest in copepod feeding.

On these grounds, it appears that the choice and selection of a specific predatory tactic is correlated with the swimming behaviour adopted. However, a copepod can switch from one strategy to another depending on the prey encountered, so as to maximise the probability of successful captures (Landry, 1980; Saiz & Kiørboe, 1995; Kiørboe *et al.*, 1996).

Once entrained in the feeding current or captured through a feeding bout, a prey is handled by the rapid motion of the second maxillae and directed towards the mouth in a predetermined alignment (Koehl & Strickler, 1981; Paffenhöfer *et al.*, 1982; Cowles & Strickler, 1983; Price *et al.*, 1983; Yule & Crisp, 1983; Buskey, 1984). Different prey

will be differently handled, as a function of their size (Price *et al.*, 1983). The food item is then tasted by the chemoreceptors present on the mouthparts (Friedman & Strickler, 1975; Friedman, 1980) and eventually rejected if considered not palatable (Friedman & Strickler, 1975; Paffenhöfer *et al.*, 1982; Bundy *et al.*, 1998), further confirming the presence of an active selection rather than a passive filter mechanism.

In summary, copepod feeding processes are varied and involve behavioural adaptations dependent on the swimming activity of the animal, as well as on the modality of interaction with the prey. Dagg & Turner (1982) estimated that 50% of marine phytoplankton in the Western Atlantic is daily grazed upon by copepods, even though the impact of microzooplankton might be important as well (Calbet, 2001). The analysis of predator-prey interactions therefore provides useful details not only on the mechanisms by which an organism reacts and interacts with its surrounding environment, but also on the effect of copepod grazing on the functioning of the pelagic food web.

3.2 Studying *Clausocalanus furcatus*

Mazzocchi & Paffenhöfer (1998 and 1999) provided a first account on the feeding habits and rates of *C. furcatus*. However, the mechanisms adopted by *C. furcatus* in the detection and capture of prey items are still unknown. The present work will highlight some peculiarities noticed for the small calanoid investigated, especially in comparison with the feeding strategies commonly reported in other copepods.

To reconstruct the capture area of the copepod, the same data set used for the analyses discussed in Chapter 2 was examined, taking into account only those sequences in which the copepod swam in the vicinity of one or more prey, either capturing it or not. The prey was represented by the dinoflagellate *Gymnodinium*

nelsoni ($55 \times 41 \text{ } \mu\text{m}$, $41700 \text{ } \mu\text{m}^3$), at a concentration of $0.2 \text{ mm}^3 \text{ l}^{-1}$ (Mazzocchi & Paffenhöfer, 1999).

Using the same code implemented by "e-voluzione" (s.r.l., Napoli) for the swimming trajectories (see § 2.2.1), the position of the rostrum and of the tips of the first antennae of the copepod, as well as that of the prey, were recorded for each frame of the clip. When more than one prey was present, the coordinates of all of them were digitised. Digitising all prey available, independently of capture, allows discrimination of the position of seized cells with respect to the ignored ones. This permits the reconstruction of the geometrical extent of the capture area of *C. furcatus*, as well as the analysis of the mechanisms involved in the perception of the prey.

In this analysis, all recognisable captures have been selected for a total of 42 events, without discriminating between the sequences on the basis of their duration. In fact, the focus of this part of the thesis work was to investigate which was, if any, the preferential geometrical arrangement for a successful interaction between *C. furcatus* and its prey. Therefore, also the clips in which the copepod was in focus for a few frames could be extracted and analysed.

Following Gerritsen & Strickler (1977), a capture was considered successful only when it was followed by the ingestion of the food particle. This was verified by analysing the frames following that of the capture: if the dinoflagellate disappeared from the recording, the capture was considered successful; by contrast, if the prey reappeared a distance away from the position in which it had been seized, it was considered as rejected.

Once digitised, the coordinates have been rotated to create a "copepod-centred reference frame" (henceforth CCRF): in this new reference frame, the origin overlaps with the rostrum of *C. furcatus*, its first antennae lying along the x-axis. The CCRF can be considered as an intrinsic reference frame (Beatty, 1986), i.e. a frame which is not fixed in space but "follows" the item of interest. The CCRF allows visualising the position of the prey with respect to the head and to the antennules of the copepod, thus showing the geometrical arrangement of captured versus non-captured particles.

An index of efficiency of capture (EC) has been measured, given by:

$$EC = \frac{\text{n}^{\circ} \text{ of captured prey}}{\text{n}^{\circ} \text{ of total items encountered}} \times 100$$

This parameter allows a calculation of the probability that an encounter can result in a successful capture, thus representing a proxy of the feeding performance of the copepod.

3.3 Mechanisms and geometry of capture in *C. furcatus*

From the entire data set, 53 clips were extracted, showing the copepod swimming in presence of one or more prey items. A total of 111 potential interactions were highlighted; an interaction is indicated here as the presence of one prey item in the close vicinity (i.e. <1mm away from the rostrum) of the copepod. Details on each clip utilised are given in Appendix II. Differently from the trajectories analysed in Chapter 2, in this case all the clips of interest were tracked independently on the number of frames they were made of.

Of the 111 interactions digitised, only 42 resulted in a successful capture. The overall efficiency of capture was equal to 38.2%. This indicates that the EC for *C. furcatus* is very low, confirming the low feeding rates reported by Mazzocchi & Paffenhöfer (1998 and 1999). A total of 69 unsuccessful interactions, in which the copepod did not attempt to grab a dinoflagellate, were recognised from the same video recordings.

C. furcatus does not create feeding currents, nor does it display raptorial behaviour. All captures are performed by direct interception of the prey, i.e. when the food items are aligned along the anterior-posterior axis of symmetry of the predator (Figure 3.1), in proximity to its head. On capturing a quarry, *C. furcatus* does not change its motion but maintains its swimming behaviour and speed with the first antennae wide open. Only on a few occasions, the copepod flicked the antennules

rapidly, but this did not appear to be associated with the feeding event. No multiple catches were observed, even when two or more cells were very close together and all close to the rostrum. No feeding activity was seen during the occasional hops, the somersaults and the fast straight displacements that occasionally interrupt the convoluted natatory activity (as discussed in Chapter 2).

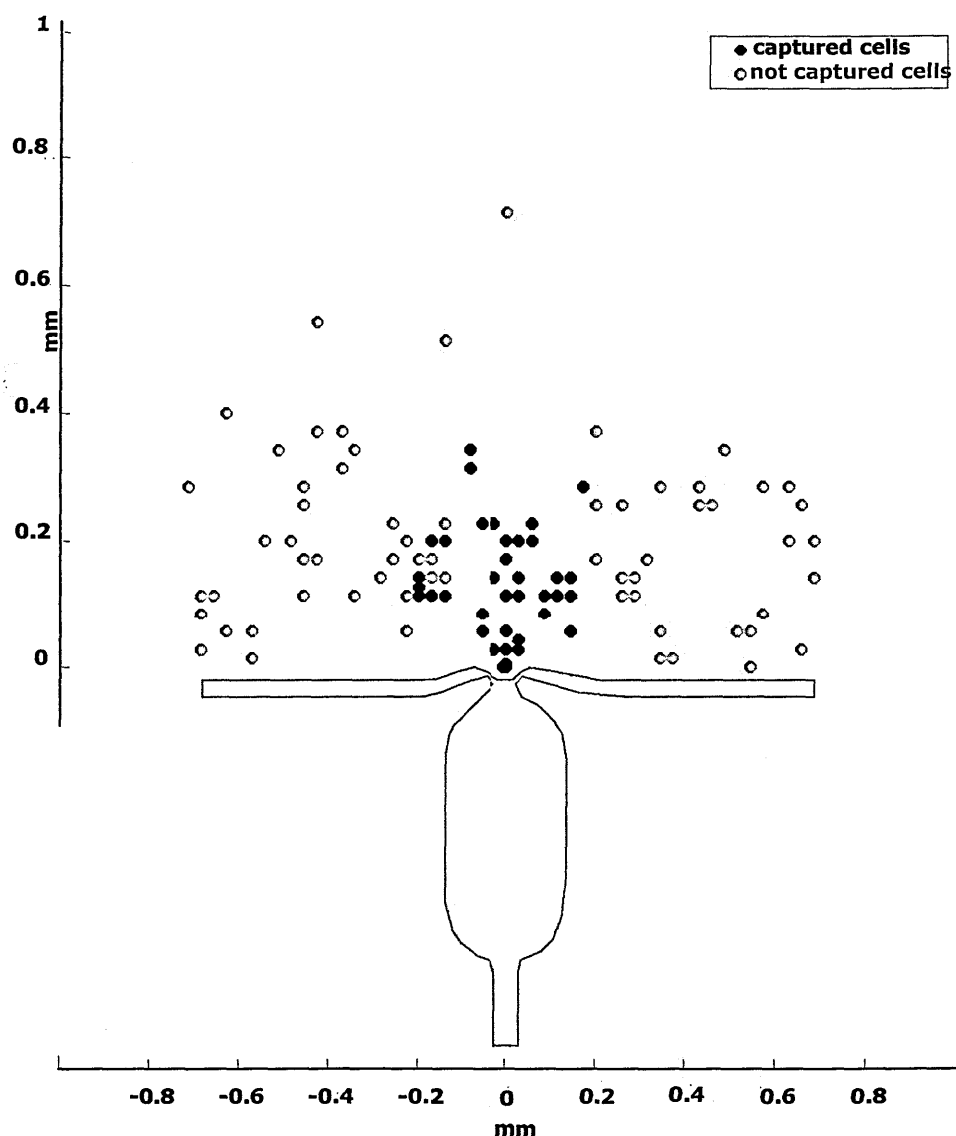


Figure 3.1: two-dimensional pattern of captures in the copepod-centred reference frame. All captures are concentrated in a rather restricted area frontal to *C. furcatus*. Prey and copepod not in scale.

Figure 3.1 shows the geometrical arrangement of captured and not-captured prey items in the CCRF. For captured prey, the position immediately preceding the capture (one frame, 1/60 s) before grabbing is reported, whereas for not-captured cells the position closest to copepod's rostrum is considered. It is worth noticing that in the plane of reference the position of some prey overlapped, therefore the number of items reported in the schematic is less than the number of interactions investigated (111). In some sequences without capture, the dinoflagellate cell appeared to be alerted by the presence of the approaching copepod and moved away from it.

The analysis of the capture events show that all catches occur in an area placed frontally to the copepod (Figure 3.1), extending 0.4 mm frontally and 0.2 mm laterally to the head of the specimen. This results in a capture area of 0.16 mm² with an EC=84%; outside this area no capture takes place (EC=0%).

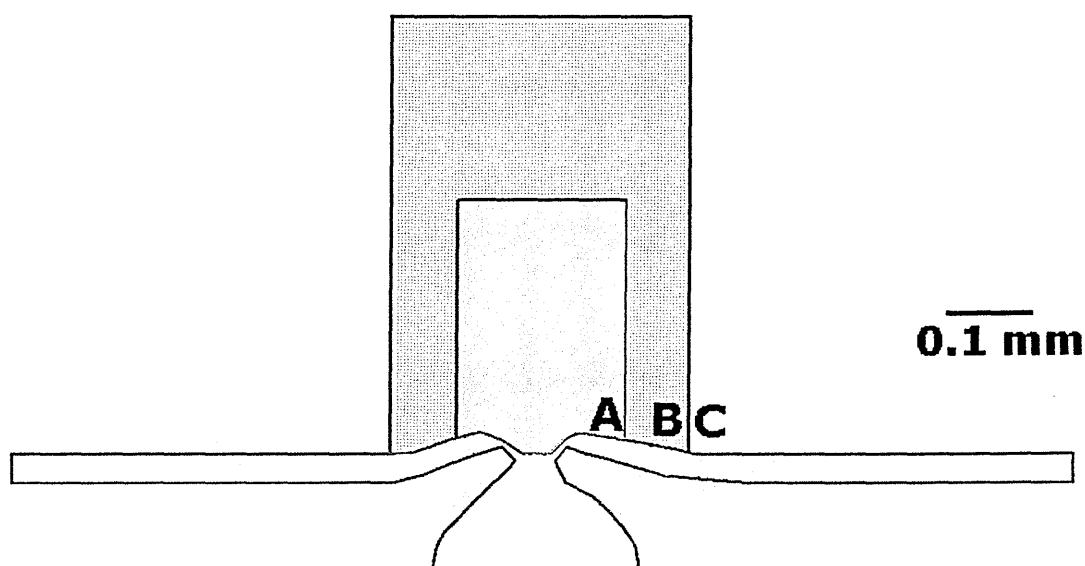


Figure 3.2: diagram showing the predatory cores identified for *C. furcatus*. A: inner core; B: medium core; C: outer core.

Looking at the pattern of capture, it is possible to identify three regions or "cores" (equivalent to those described by Strickler, 1985, for copepods' feeding currents) (Figure 3.2), which can be discriminated on the basis of their efficiency of capture. The inner core (A) is a narrow corridor (approximately 0.06 mm²) with the lower side

centred on copepod's rostrum, characterised by the highest EC (100%). This area is surrounded by an intermediate core (B) with EC=62%; the capture area results from the superimpositions of cores A and B. In the outer core (C), no capture occurs (EC=0%), independently on the distance from the rostrum of the copepod. It is worth recording here that all video recordings have been performed under the same food concentration, thus excluding any dependence of the perception distance on prey availability (Paffenhöfer & Lewis, 1990). Moreover, it is important to consider that, even though the copepod does not seize some cells in region B and any item in region C, this does not necessarily imply that they are not sensed by *C. furcatus*.

On some occasions, *C. furcatus* seemed to loop around a cell located near the tip of the A1 until it was in a favourable position for a capture (inside cores A or B). It is however not possible to assess that this behaviour represented a specific feeding strategy of *C. furcatus*.

Once captured, the prey is handled by copepod's mouthparts and presumably tasted by the chemoreceptors present on them, as reported for other species (Friedman & Strickler, 1975; Friedman, 1980; Bundy *et al.*, 1998), eventually being rejected if not palatable. In agreement with the observations by Price & Paffenhöfer (1985) on *Eucalanus pileatus*, the handling of the food particle is performed by the rapid movement of the feeding appendages of *C. furcatus* soon after the capture, as evident in some sequences in which the copepod was observed from the lateral view.

3.4 Discussion

Perceiving and capturing a prey results from a proper localisation and identification of the item, and from a successful predatory habit. Whilst for the first point a major role is played by the mechano- and chemo-sensory abilities of the copepod, for the

second issue the swimming behaviour has important consequences on the dynamics of capture.

The analysis of the video recordings provides important details on the mechanisms of interaction between *C. furcatus* and its prey, permitting the reconstruction of its capture area. Basing on the results obtained, it is possible to state that *C. furcatus* captures only those dinoflagellates placed within a characteristic area, whereas all prey located outside the capture area are out of reach, independently of the distance from the copepod's A1s. In no occasion, neither during the characteristic cruising nor while displaying other swimming behaviour (see § 2.3), *C. furcatus* has created feeding currents. Currents created by the feeding appendages were noticed only in narcosis-recovering specimens. When the effect of the drug was disappearing, *C. furcatus* started moving its appendages with a frequency lower than that used while cruising; this resulted in the creation of a typical funnel-shaped current. However, as soon as the effect of the narcotic had ceased, the beating appendages re-attained their characteristic frequency; in such condition, currents could no longer be recognised. It is therefore evident that the motion of the copepod itself does not allow it to exploit this widespread feeding strategy, but it has had to evolve alternative mechanisms of capture.

The feeding strategy of *C. furcatus* presents marked differences with the predatory behaviour discussed in § 3.1. While ambush predators maximise their capture areas by attacking prey that are perceived by their mechanoreceptors (e.g., *Oithona similis*: Svensen & Kiørboe, 2000; *O. plumifera*: Paffenhöfer & Mazzocchi, 2002), *C. furcatus* presents a small capture area especially if compared to the extension of its antennules. Moreover, *C. furcatus* never performs feeding bouts but collects prey only via direct interception: if a prey is outside the capture area, the copepod will not seize it.

Other marked differences arise from the comparison between the feeding behaviour of *C. furcatus* and the scheme of a typical feeding current. The small calanoid does not create such structured flow, probably owing to its own swimming

behaviour. This, in fact, is characterised by frantic propulsion which might prevent the copepod from using its appendages to create a feeding current. In fact, copepods adopting such predatory behaviour show smoother natatory activity, (e.g., Bundy *et al.*, 1993; see, for example, a movie from Malkiel *et al.*, 2003, available at the URL: <http://jeb.biologists.org/content/vol206/issue20/images/data/3657/DC1/Movie1.mov>) In addition, while feeding currents convey particles from a wide region towards the mouthparts, *C. furcatus* has potentially successful interactions only with those prey located inside its relatively limited capture area.

C. furcatus has a reduced two-dimensional capture area. Compared to that of other swimming copepods displaying active predatory behaviour (e.g., *Cyclops*: Kerfoot, 1978; *Euchaeta rimana*: Doall *et al.*, 2002), the capture area of *C. furcatus* is up to ten times smaller and limited to a restricted, almost rectangular region before copepod's head. On the contrary, in the case of *Cyclops* (figure 4.c in Kerfoot, 1978) the capture area is triangular-shaped (the vertex overlapping with copepod's rostrum) with a curvilinear base, bringing to an enlargement of the attack region. Moreover, in *C. furcatus* none of the dinoflagellates located outside this predatory core was preyed upon, even if very close to the antennule. This scenario markedly contrasts with the predatory behaviour of *E. rimana* (figure 2.a in Doall *et al.*, 2002), which is instead capable of attacking and capturing quarries located in correspondence of all segments of the A1. The shape and limited extension of the capture area of *C. furcatus* suggests that this copepod is not able to detect distant items, but seems fitted for the collection of food items close to its head.

The looping behaviour of *C. furcatus* has no direct effect in the displacement of particles. Video recordings of specimens swimming in presence of passively buoyant artificial lattice beads (20 μm \varnothing ; Coulter Corporation, USA) show that the distribution of the particles is not modified by the movement of the copepod. This is likely due to the high viscosity of the medium, which prevents the particles to be dislocated despite the frantic swimming motion of *C. furcatus*.

The data utilised in the present work are strictly two-dimensional, allowing only the reconstruction of the capture area of *C. furcatus*. At present no information is available on the three-dimensional arrangement of the capture volume. Basing on current results, it is reasonable to hypothesize that the copepod would exploit a rather constricted parallelepiped-like volume, still centred on its head but with a reduced height.

As well as the swimming behaviour (Chapter 2), also the feeding strategy adopted by *C. furcatus* represents an unusual example among copepods. These natatorial and predatory mechanisms are likely to be strictly associated. Owing to its typical fast propulsion, the copepod does not seem able to veer rapidly enough to perform feeding bouts towards prey located even just outside the capture area. Such attack would in fact consist of the following steps:

- identification and localisation of the quarry
- reorientation towards the prey
- bout and capture

which occur over times of 50-100 ms (Bundy *et al.*, 1998; Doall *et al.*, 2002; Paffenhöfer & Mazzocchi, 2002). While this behaviour is usually utilized by cruising copepods such as *Diaptomus sicilis* (Bundy *et al.*, 1998) and *Euchaeta rimana* (Doall *et al.*, 2002), it has never been observed in the video recordings of *C. furcatus*.

As proposed by Mazzocchi & Paffenhöfer (1999) and as already discussed in § 2.4, *C. furcatus* seems to explore small volumes of fluid in rapid succession. This swimming activity, coupled with the feeding strategy depicted from the current results, suggests that copepod swimming and feeding behaviour might be optimised for capturing patchily distributed prey. In such a situation, in fact, the small calanoid may raid into the cluster and sweep it out just by swimming across it. By travelling fast, *C. furcatus* may rapidly exploit the patch, reducing the possibility of cluster disgregation. In addition, by moving through the patch, the predator will increase the probability of finding a prey inside its capture area, thus maximising feeding success.

Chapter 4

**Sensory structures and performance in
*Clausocalanus furcatus***

4.1 Sensing the environment

Animals modify their behaviour in response to the information gained from their surroundings (Kamil, 1988). In particular, they perform choices about feeding, mating and predator avoidance, on which their own survival depends, on the basis of the type and strength of the signals perceived from the environment (Kamil, 1988).

As almost all other crustaceans, copepods rely on both mechanical and chemical stimuli to gain information from their surrounding environment (Laverack, 1968; Ache, 1982; Bush & Laverack, 1982). The detection of external cues is assured by different receptors which, in Laverack's (1968) words, "accept energy of one type (mechanical, light, chemical) and transform it to another (electrical)"; the role of these receptors is therefore to convey to the central nervous system (Park, 1966; Marshall & Orr, 1972) a signal alerting the animal to what is occurring around it.

Copepods possess numerous sensors on different parts of their body, but mainly along their antennules. The first antennae (A1) are composed of a varying number of segments, usually 27 in Calanoida (Huys & Boxshall, 1991). They derive from the A1 of the ancestral copepod, presumably made up of 28 segments (Boxshall, 1983; Boxshall *et al.*, 1984), with respect to which some elements are compound or "fused" due to either a failure in separation or to secondary fusion (Boxshall & Huys, 1998). The total number of expressed segments may furthermore be utilised to infer the degree of evolution of the species considered, multi-segmented antennules being more primitive than those with a higher number of fusions (Boxshall *et al.*, 1984; Huys & Boxshall, 1991; Schutze *et al.*, 2000). The role played by the A1s and by the sensory structures present on them in the detection of external stimuli has been demonstrated by ablation experiments (Mullin & Brooks, 1967; Landry, 1980; Gill & Crisp, 1985), direct stimulation (Gill, 1985; Fields & Yen, 1996; Field & Yen, 2002) and electrophysiological recordings (Yen *et al.*, 1992; Gassie *et al.*, 1993; Lenz & Yen, 1993; Hartline *et al.*, 1996; Fields *et al.*, 2002; Fields & Weissburg, 2004).

Setae are the most common supracuticular mechanoreceptors found in crustaceans (Watling, 1989). A seta is "an articulated cuticular extension" (Watling,

1989) with a 9+0 basal body structure (Strickler & Bal, 1973; Yen *et al.*, 1992; Crouau, 1997), involved in the detection of mechanical stimuli (Laverack, 1968). Their cuticle is generally thick and they are set on a flexible base inside a socket articulation; they may either move in all directions or be directionally-sensitive, depending on the characteristics of the basal innervation and/or of the morphology of the socket itself (Bush & Laverack, 1982). Setae with different morphotypes (Factor, 1978; Bush & Laverack, 1982) are associated with specific functional roles (Jacques, 1989; Yen *et al.*, 1992); moreover, apical pore bearing setae are presumably involved in the dual detection of mechanical and chemical stimuli (Jacques, 1989). Copepods are sometimes also equipped with other supracuticular receptors, the so-called pegs, are articulated modified hairs barely projecting above the cuticle (Blades & Youngbluth, 1979; Bush & Laverack, 1982; Malt, 1983; Gill, 1986; Kurbjeweit & Buchholz, 1991).

Among crustaceans, copepod setae are the most responsive and sensitive (Weatherby & Lenz, 2000; Fields & Yen, 2002), reacting to a wide range of frequencies (from 30 Hz up to 5 kHz) (Yen *et al.*, 1992; Gassie *et al.*, 1993; Fields & Weissburg, 2004). Reaction times are in the order of ms, with species-specific values (Yen *et al.*, 1992; Lenz *et al.*, 2000; Fields & Weissburg, 2004); the fastest responses are reported for those copepods, among which are the Clausocalanidae, bearing myelin-like sheaths in their axons (Davis *et al.*, 1999; Lenz *et al.*, 2000; Weatherby *et al.*, 2000). Unlike other biological processes (e.g., respiration, growth, etc.), neurophysiological responses show a low dependency on temperature, without remarkable differences between high and low temperatures (Lenz *et al.*, 2005).

The disposition of the setae along the antennular structure is indicative of the modality of cue detection and appears to be correlated with the swimming activity of the copepod (Paffenhöfer, 1998). Of the entire A1, the distal tip represents the most sensitive area (Gill, 1985; Yen *et al.*, 1992; Lenz & Yen, 1993; Fields *et al.*, 2002; Fields & Weissburg, 2004), bearing the longest setae and acting as an early-warning system (Lenz & Yen, 1993).

Mechanoreception takes part in the detection of prey (e.g., Landry, 1980; Buskey, 1984; Fields & Yen, 2002), predators (e.g., Lenz & Hartline, 1999; Fields & Yen, 2002) and mates (e.g., Strickler, 1998; van Duren *et al.*, 1998; Yen *et al.*, 1998). Despite such a key role, the process of signal detection is still poorly understood. Légier-Visser *et al.* (1986) state that copepod mechanoreceptors act as pressure detectors, similar to fish calcareous otoliths. This scheme has been criticised (Visser, 2001; Yen & Okubo, 2002), and a velocity-based model (Kjørboe *et al.*, 1999; Kjørboe & Visser, 1999; Visser, 2001) and a fluid deformation hypothesis (Yen & Okubo, 2002) have been proposed.

Also chemoreception is crucial for copepods' sensing of the environment, even though crustacean chemosensitivity is not well understood (Ache, 1982). Chemical sensors are distributed along the entire body, for example on the mouthparts (Ong, 1969; Friedman & Strickler, 1975; Friedman, 1980) where they check for the palatability of food particles. Antennular chemosensitivity is ascribed to aesthetascs (Ache, 1982), key elements in both marine and terrestrial crustaceans (Ghirardella *et al.*, 1968), formed by the aggregation of a large number of chemoreceptors (Laverack, 1988) and acting as organs of smell (Ache, 1982).

Aesthetascs are thin-walled (Laverack & Ardill, 1965), tubular sensory filaments (Huys & Boxshall, 1991), recognisable by a spongy cuticle and a characteristic neck constriction at their base (Ache, 1982; Heimann, 1984; Kurbjeweit & Buchholz, 1991; Bundy & Paffenhöfer, 1993). The sponge-like external structure, rather than an eventual apical pore, is highly likely to be responsible for instantaneous odorant absorption from the surrounding medium (Shelton & Laverack, 1970; Ache, 1982; Heimann, 1984; Lenz *et al.*, 1996). The most commonly reported aesthetasc distribution over copepods' A1s shows a maximum density on the first segments (Boxshall *et al.*, 1997; Moore *et al.*, 1999), where the flow of the feeding currents is stronger thus increasing the efficiency of odour perception (Jackson & Kjørboe, 2004).

Chemoreception has long been recognised to be a primary actor in the communication between a mating couple (e.g., Katona, 1973; Griffiths & Frost, 1976;

Doall *et al.*, 1998; Tsuda & Miller, 1998; Weissburg *et al.*, 1998; Yen *et al.*, 1998), but it is also fundamental for the detection of feeding stimuli (e.g., Poulet & Marsot, 1978; Poulet & Ouellet, 1982; Buskey, 1984; Gill & Poulet, 1988), for predator avoidance (e.g., Folt & Goldman, 1981) and possibly for host recognition in symbiotic species (as discussed in Ache, 1982).

Some copepods bear integumental pores (Blades & Youngbluth, 1979; Malt, 1983; Gill, 1986; Kurbjewit & Buchholz, 1991; Weatherby *et al.*, 1994), with a species-specific signature pattern that has taxonomic value (Malt, 1983; Park, 1995 and references therein). Pores can be morphologically different (Blades & Youngbluth, 1979; Gill, 1986; Weatherby *et al.*, 1994) and are usually associated with other sensilla (Malt, 1983; Gill, 1986; Kurbjewit & Buchholz, 1991; Weatherby *et al.*, 1994). The role of these structures is still rather unknown: they apparently have a secretory function (Blades & Youngbluth, 1979; Weatherby *et al.*, 1994), even though a sensory capability is plausible as well (Gresty *et al.*, 1993).

The analysis of the sensors present on and of their pattern of distribution along the antennule provides a first indication of the perceptive performance of the copepod. This latter aspect can be complemented with neurophysiological experiments recording the nerve impulses associated with the stimulation of the sensory organs. To this aim, part of this work has concentrated on the morphology and the sensitivity of the first antennae of *C. furcatus*. The morphological analysis, performed utilising different complementary approaches, indicates which are the structures involved in the acquisition of the cues. The integration of these results with electrophysiological experiments on the perceptive performance of *C. furcatus* in the presence of prey-mimicking mechanical stimuli allows a more complete picture of the mechanisms utilised by this small calanoid to detect potential food items to be presented.

4.2 Morphological analyses

Morphological analyses are useful to understand which are the sensory structures present on and what is their disposition along copepods' antennules. Such information provides a first insight into the mechanisms and signals used by this copepod to collect information from the surrounding environment.

A detailed morphological investigation of the A1s of *C. furcatus* has been carried out utilizing four different methodologies: camera lucida drawings, scanning electron microscopy, transmission electron microscopy and laser scanning confocal microscopy, each focusing on some peculiar features of the structure.

4.2.1 Terminology adopted

The terminology utilised in the following sections adheres to the most commonly adopted schemes found in the literature. In particular:

- segment numeration follows Huys & Boxshall (1991), with Roman and Arabic numerals identifying ancestral and actual segments, respectively;
- setal morphotypes is based on the schematization by Factor (1978);
- antennule edges are labelled following Gill (1985), as shown in Figure 4.1.

4.2.2 Copepod sampling and preservation

All specimens utilised for morphological investigations came from zooplankton samples gently collected by vertical hauls using a Nansen net (200 µm mesh size), using a 5 l glass jar as a cod-end bucket, in the upper layer (0-70 m) of an offshore station in the Bay of Naples (Tyrrhenian Sea).

The jars were placed in a cooler and rapidly brought to the laboratory in a temperature controlled room, set equal to the depth-averaged temperature of the sampled water column. The samples were diluted using seawater collected from the sampling site, and adult females of *C. furcatus* were sorted using a large-mouth glass pipette. The recognition of the individuals was initially performed on the basis of their

swimming activity; subsequently, specimen integrity was further checked under a stereoscope (Leica MZ12-5). If individuals were not immediately used for morphological analyses, they were fixed and preserved in 5% buffered formalin solution.

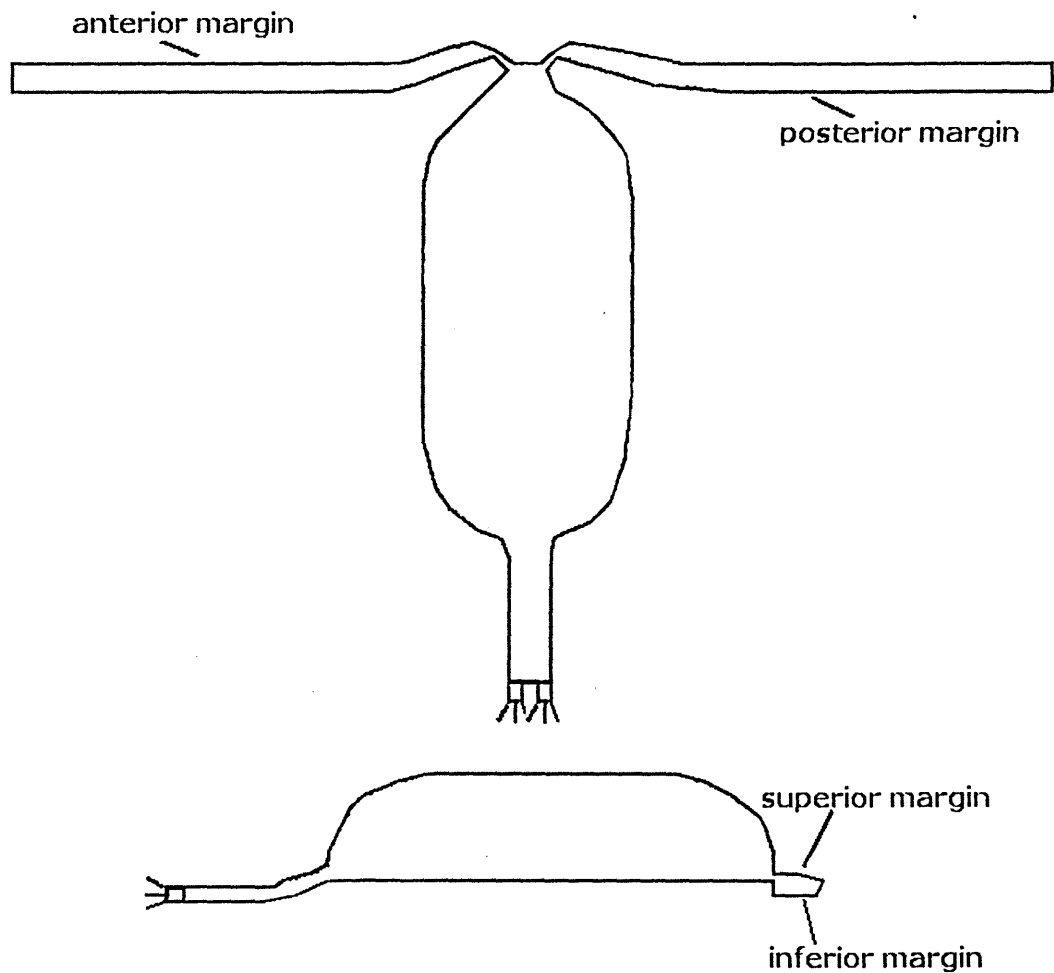


Figure 4.1: schematic showing the terminology utilised for antennule edges; terminology based on Gill (1985).

4.2.3 Camera lucida drawings

Camera lucida drawings (henceforth CLDs) give a synthetic description of the gross external morphological features of the A1s, showing the patterns of segmentation and setation.

Sorted adult females of *C. furcatus* were narcotised with MS222 (tricaine methane sulphonate) (1 g l^{-1}) (Mullin & Brooks, 1967; Omori & Ikeda, 1984) and placed on a slide; their total length and that of their A1s were measured under a dissecting microscope (Leica MZ12-5) equipped with an ocular micrometer. Total body length was measured as the distance between the anterior margin of the rostral area and the posterior margin of the caudal rami (Böttger-Schnack, 2001).

The antennules were manually dissected at the base of the A1 using a couple of needles, following the procedures of Huys & Boxshall (1991). The antennules were mounted on a permanent slide in distilled water enriched with some droplets of glycerine, so as to make the sample more easily visible. A cover slip was mounted and glued with transparent nail varnish. The position of the detached antennule was marked on the cover slip using a permanent-ink marking pen.

CLDs were performed using a Zeiss Axioshop 2 plus microscope equipped with a Zeiss Axioshop camera lucida tube. The entire antennule was drawn at 20X and 40X magnifications, whereas the distal tip was further analysed at 100X.

4.2.4 Scanning electron microscopy

Scanning electron microscopy (SEM) is a well established technique by which to investigate micro-scale external morphological characteristics of an object (Heywood, 1971). SEM has often been utilised to study the disposition and typology of receptors along copepods' A1s (e.g., Strickler & Bal, 1973; Gill, 1986; Weatherby *et al.*, 1994), as well as in the identification of ultrastructures such as pores (e.g., Malt, 1983) and pegs (e.g., Gill, 1986).

This technique allows reconstruction of the three-dimensional pattern of disposition of sensors along the entire antennule. In addition, through its very high resolution properties, SEM permits investigation of the microscale morphological structures of the sensors. However, during specimen preparation some structures may be damaged and eventually lost.

To remove external particles (e.g., salt ions, detritus, food items), copepods were preliminary rinsed twice (30 min per wash) in Millipore Milli-Q plus bidistilled water (BDW) filtered on a Millipore Millex-GS 0.22 μm membrane. Individuals were subsequently dehydrated in a graded ethanol series (Table I) using 100% ethanol (J. T. Baker, Holland) filtered on a Whatman 3.0 μm polycarbonate membrane and BDW (filtered as above), following a modification of the procedure reported by Huys & Boxshall (1991).

Individuals were critical point dried from liquid carbon dioxide (5 h), mounted on an aluminium stub and platinum coated. Observations and photographs were performed using a Philips SEM 505 and a Jeol JSM-6700F.

Table I: graded ethanol series used in the preparation of specimens for SEM analysis (modified from Huys & Boxshall, 1991).

Ethanol series	Duration
30%	30 min
50%	30 min
70%	30 min
80%	30 min
80%	30 min
90%	30 min
90%	30 min
100%	30 min
100%	30 min
100%	overnight

4.2.5 Transmission electron microscopy

Transmission electron microscopy (TEM) allows determining the finest details of internal structure of materials. TEM pictures are two-dimensional images of ultra-thin section of the material analysed; in the case of biological samples, TEM highlights cell structure and morphology, with magnifications up to 350,000 times. TEM has already been utilised for the analysis of copepods' antennular sensors (Kurbjeweit & Buchholz, 1991; Weatherby *et al.*, 1994; Lenz *et al.*, 1996; Weatherby & Lenz, 2000; Weatherby *et al.*, 2000), providing useful details to discriminate between the different structures.

In the present thesis, specimens were prepared following a modification (Tina Weatherby Carvalho, Biological Electron Microscope Facility-University of Hawaii at Manoa, *pers. comm.*) of the procedure described in Weatherby *et al.* (1994). Specimens collected from a live sample were preliminarily sedated using MS222 and checked for the integrity of their A1s. Selected females were prepared according to the steps listed in Table II.

Table II: chemical fixation for TEM analysis.

Process	Duration
fixation in 4% glutaraldehyde + 0.1M NaCAL + 0.35M sucrose (pH=7.6) [primary fixative]	15-30 min
decalcification in 2% disodium EDTA added to the primary fixative	1-1.5 h
primary fixative	30 min
wash in NaCAL 0.1M + 0.44M sucrose	2x10-15 min
post-fixation in 1% OsO ₄ in 0.1M NaCAL	1 h
wash in 0.1M NaCAL	15 min
dehydrations in ethanol series (50%-70%-85%-95%)	2-3x3-5 min
dehydration in 100% ethanol	3x10 min
propylene oxide	3x10 min
1:1 propylene oxide:epoxy resin	overnight
100% epoxy resin	2-8 h
100% epoxy resin	1-4 h
polymerisation in oven (60°C)	2-3 d

Ultrathin section (500 Å) (Leica Ultracut UCT) were taken throughout the entire length of the A1 of several specimens. Sections were double-stained with uranyl acetate and lead citrate, viewed and photographed in a Zeiss LEO 912 AB.

4.2.6 Laser scanning confocal microscopy

Compared to the techniques reported above, laser scanning confocal microscopy (LSCM) has been applied to copepods in relatively recent times (Bundy & Paffenhöfer, 1993; Carotenuto, 1999; Carotenuto *et al.*, 2002). LSCM is efficient in targeting and discriminating mechano- and chemoreceptors (Bundy & Paffenhöfer, 1993), as well as muscular bands and other anatomical details (Carotenuto, 1999; Carotenuto *et al.*, 2002). LSCM reduces the risks of sample damage typical of SEM preparation: sensillae are rapidly stained by a fluorescent carbocyanine dye (DiI), which penetrates into the lipid bilayer of neuronal cell membranes without spreading between neurons (Honig & Hume, 1986). On the other hand, LSCM does not allow for investigation of the microscopic characteristics of the sensors analysed, as is possible using SEM.

Preparation of the specimens was performed following a modification of the protocol presented in Carotenuto (1999), which was in turn based on Bundy & Paffenhöfer (1993). Individuals were initially rinsed twice for 5 minutes in a 1:1 solution of BDW and filtered sea water (FSW), both further filtered on a Millipore Millex-GS 0.22 μm membrane. Subsequently, the sample was rinsed three times (for a total wash of 5 min) in BDW only. Specimens were then dehydrated in a 50% (2 min) and in a 70% (5 min) ethanol solution. After that, they were incubated for 1 h in dark conditions in a 70% ethanol solution enriched with 4 drops of DiI (Molecular Probes, Inc.) for each ml of solution (see Carotenuto, 1999, for details on DiI preparation). At the end of the staining, the sample was rinsed in 50% ethanol for 1 min, then three times in BDW for a total of 15 min. Differently from Carotenuto (1999), the final step of the preparation was performed using BDW rather than FSW in order to avoid deposition of salt ions on the surface of the organisms, which might interfere with the observations (I. Buttino, Stazione Zoologica di Napoli "Anton Dohrn", *pers. comm.*).

The specimens were placed on a thin glass slide and covered with a cover slip. The observations were performed using a Zeiss LSM 510 Meta ($\lambda=543\text{ nm}$) at 100X magnification. The microscope was connected to a PC running specifically conceived software (Zeiss LSM 510). Images of the different segments of the antennules were

collected on consecutive focal planes, digitally stored and either compiled or analysed as single optical sections (thickness: 1 μ m). Detector gain, amplifier offset, pinhole and laser excitation have been set to maximise the response from antennular sensors and muscles, while at the same time minimising the intensity of the autofluorescence of the chitinous structure of the A1.

4.3 Neurophysiological analyses

Electrophysiological experiments allow the sensitivity and neurophysiological performance of the mechanoreceptive sensory sensilla located on copepods' antennules to be estimated. Such results are of paramount importance to quantitatively estimating the reaction of a specimen, in terms of traffic nerve impulse, to mechanical cues originating from the surroundings. Such information complements morphological investigations, since it is possible to associate specific responses to the different sensors stimulated.

4.3.1 Experimental set-up

The experimental set-up utilised for neurophysiological experiments was a modification of Gassie *et al.* (1993) (Figure 4.2). A small plastic cell culture dish (35x10 mm, diameter x depth) (Corning Inc., U.S.A.) was partly filled with a silicone elastomer (Sylgard- Dow Corning, Germany); the plastic bottom was then carved so as to create a small pool (observation chamber) (volume: ~ 1.5 ml) in the centre of the dish. One-third of this volume was filled with mineral oil (Carlo Erba, Italy) made denser by mixing it with solid vaselin; seawater was poured in the remaining part of the chamber (~ 1 ml).

An adult *C. furcatus* female was clamped on the urosome by means of stainless steel forceps (FST, U.S.A.), the aperture of which was regulated by means of a silicon o-ring sliding on the tips. The forceps were mounted on a micromanipulator (Prior,

England). The copepod was gently drawn into the oil almost entirely, with one of its A1s only protruding in the seawater. A silver electrode was placed in the seawater, whereas a second electrode was connected to the forceps. Standard extracellular recording techniques (Fields *et al.*, 2002) were utilised to measure the electric potential between the two silver wire electrodes; these were connected to an amplifier, which in turn was connected to a PC. Nerve impulse traffic (hereinafter NIT), defined as single or multiple spontaneously or evoked potentials with a duration of 2-5 ms, was monitored and recorded using Clampex 9.0 (Axon Instruments Inc., U.S.A.), and data were subsequently post-processed using Clampfit 9.0 (Axon Instruments Inc., U.S.A.).

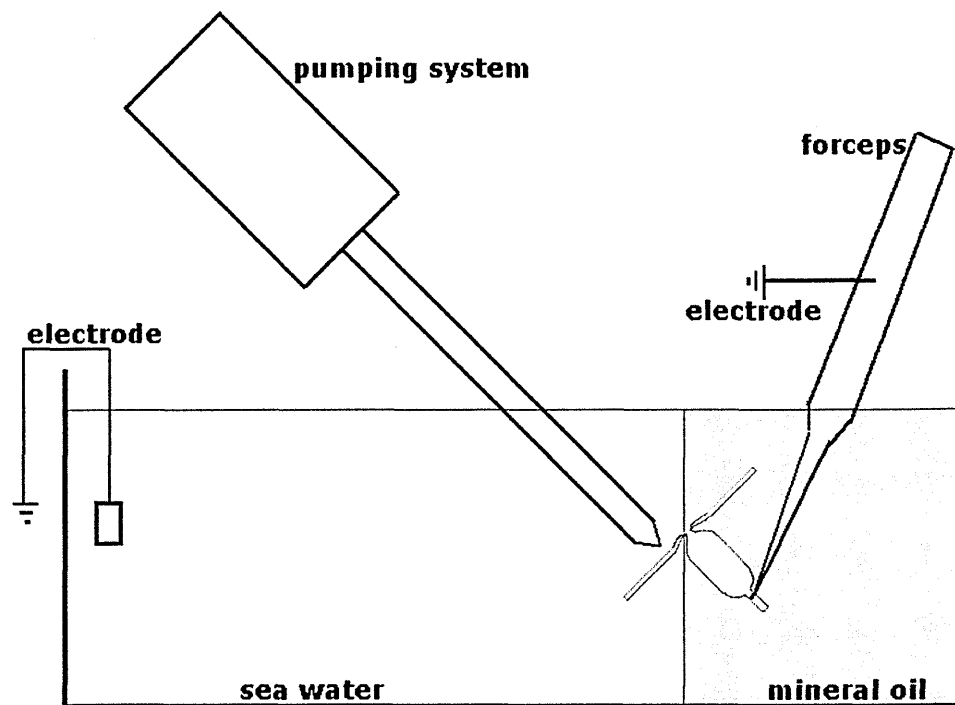


Figure 4.2: schematic of the experimental set-up utilised for the neurophysiological experiments. Elements are not in scale.

Preliminary tests were carried out to test the efficiency and the possible consequences of copepod tethering. The animal was held both on the prosome and on the urosome by gentle clutching of the forceps; however, only this second approach proved efficient. Only in this condition, in fact, could the animal resume its swimming

activity almost immediately, even when released after several hours of restraint. This result indicated that the procedure was not detrimental to the animal and would not affect its neurophysiological response.

Some authors (Yen *et al.*, 1992; Fields *et al.*, 2002; Fields & Weissburg, 2004) recorded NIT from detached antennules. Such an approach has been tested on *C. furcatus* but, owing to the reduced length of the A1 (~ 700 μm), results were not satisfactory because the small dimension hindered safe handling of the structure. Moreover, such a procedure prevents NIT recording from the entire antennule, some segments of which are left in the oil phase of the set up (see the above cited works for further details on technical aspects of the methodologies adopted).

4.3.2 Stimulation of antennules

NIT was recorded in undisturbed conditions to determine the baseline activity. Subsequently, a prey-mimicking stimulus was utilised to trigger a neurophysiological response of the antennule. The stimulus was provided by a system similar to that used in Fields & Yen (2002), in which an electronically-controlled pumping system worked as pulse generator, connected to a fine plastic tip ($\varnothing \sim 200 \mu\text{m}$) mounted on a micromanipulator (Prior, England). The plastic tip was filled with seawater, which was forcibly expelled until a meniscus formed at the mouth of the tip. A rapid switch (100 ms) of the valve controlling the system pushed the meniscus back and forth thus creating a wave-like pulsing stimulus. Such disturbance recreated the signal originating from a prey in relative motion with the copepod (Fields & Yen, 2002). Both the duration and the frequency of the pulses could be set via a PC plugged to the pulse generator. The plastic tip was placed approximately 2 mm from the surface of the antennule, so as to test the validity of the results discussed in Chapter 3 concerning the presence of a capture area.

As in Lenz *et al.* (2005), selective criteria were adopted to select the responses of a sufficient quality for analysis. Criteria adopted included: testing of the entire A1; replicates of measurements; consistent latency times. Out of 15 females tested, the

responses of four different females were accepted and utilised for a statistical comparison. For each specimen selected, three different sectors (basal, median and distal) of the antennules were separately stimulated and the associated NITs recorded, with a minimum of 10 replicates per sector, accounting for a total of 120 NIT tracks recorded. This approach permitted the sensitivity of each sector to the same stimulus to be differentiated, thus reconstructing a sensitivity map for *C. furcatus*. The number of females utilised for the final analysis is in line with published papers on this topic (e.g.: Lenz & Yen, 1993; Lenz *et al.*, 2000 and 2005). The partial data obtained for the specimens not taken into account in the analysis were in agreement with those from the four females selected.

As a control experiment, aimed at verifying that the recorded responses were associated with the stimulation of the A1 and not with mechanical artefacts, two of the females tested were also stimulated in presence of 2 μ M TTX (tetrodotoxin – Alamone Labs, Israel) (2 μ l for 1 ml of seawater). TTX is a potent non-protein marine neurotoxin (Alcaraz *et al.*, 1999) which selectively and reversibly blocks Na⁺ channel in nerves and muscle membranes by binding to the pores of ion channels. It is commonly found in nature in the flesh, viscera and skin of puffer fish (family Tetraodontidae) (Ahasan *et al.*, 2004), even though increasing evidence is being accumulated on the presence of TTX in other organisms (e.g., Freitas *et al.*, 1996; Pires *et al.*, 2002), as well as in some phytoplankton species (Kodama *et al.*, 1996). The effect of TTX on crustaceans is well known (e.g., Ache, 1982), and it has effectively been verified on copepods in a recent paper by Fields & Weissburg (2004). If recorded NITs were effectively associated with the stimulation of mechanoreceptors, in presence of the toxin the same stimulus should not result in any spike in the neurophysiological recordings.

Neurophysiological tests were performed on females caught from gentle vertical hauls (as in § 4.2.2), checked under the stereoscope for the integrity of their structures.

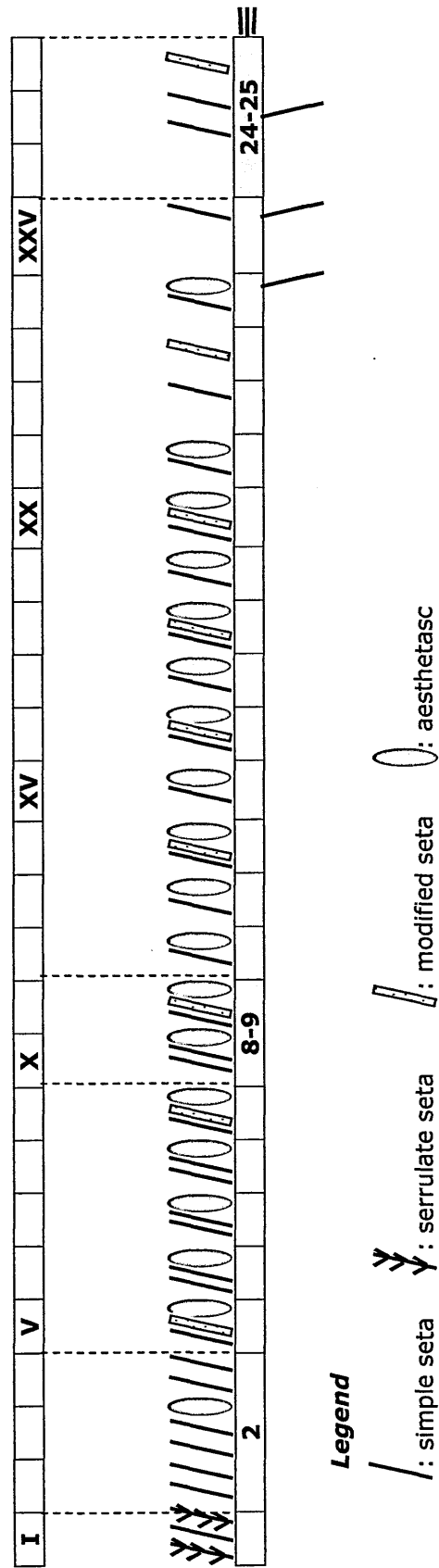


Figure 4.3: segmental homology and receptor distribution of the first antenna of an adult female of *C. furcatus*. On top of the figure, it is represented the presumed 28 segmented antennule of the ancestral copepod (Boxshall et al., 1984), identified with Roman numbers. The second schematic represents the A1 of *C. furcatus* female: actual fused segments are shaded in grey and are numbered with Arabic figures. Dotted lines show the segmental homologies between the ancestral copepod and the fused segments of *C. furcatus*. Typical trithek arrangements are shown on actual segments 3, 7, 12, 14, 16 and 18. Receptors and segments not in scale.

4.4 The antennules of *C. furcatus*

4.4.1 Morphology

Adult female *C. furcatus* have short bodies (0.99 ± 0.33 mm total body length; mean \pm standard deviation, $n=10$) with reduced antennules (0.77 ± 0.02 mm, $n=10$) comprising 25 segments, some of which are fused together (8-9 and 24-25) (Frost & Fleminger, 1968). The segmental homology, following Boxshall & Halsey (2004), is sketched in Figure 4.3. Segment 1 (I) is free, whereas segment 2 is formed by the fusion of ancestral segments II-IV. Segments 3 (V) to 7 (IX) are separated, while segments 8 (X) and 9 (XI) are fused together; segments 10 (XII) to 23 (XXV) are separated, while the apical one (24-25) is derived from the fusion of ancestral segments XXVI-XXVIII. The A1 of *C. furcatus* bears aesthetascs and three different kinds of setae: simple, serrulate and modified; all sensory structures are aligned along the anterior margin of the antennule (Figure 4.4). Following the scheme applied by Lenz & Yen (1993) to *Pleuromamma xiphias* and *Euchaeta rimana*, the antennule of *C. furcatus* can be divided into three sectors: the basal sector (segments 1 to 8-9), where setae are more closely spaced; the median sector (segments 10 to 18), where setae become sparse; and the distal sector (segments 19 to 24-25) bearing a reduced number of setae. The antennules also bear single aesthetascs on segments 3, 7, 8-9, 12, 14, 16, 18, 21 and 24-25; in almost all cases, with the exception of segments 8-9 and 21, aesthetascs are arranged in the trithek scheme (the antennulary armature comprising 2 setae and 1 aesthetasc) typical of female copepods (Boxshall, 1983; Huys & Boxshall, 1991; Kurbjewit & Buchholz, 1991; Boxshall & Huys, 1998; Miller, 2002). All tritheks reported show the particular arrangement described by Giesbrecht (as cited in Boxshall, 1984), with one seta almost in the middle of the segment, and another seta close to the aesthetasc on the distal part; in *C. furcatus* females, the seta close to the aesthetasc is always a modified one. The receptors are never arranged in quadritheks, a distinctive male arrangement sometimes reported also for some female

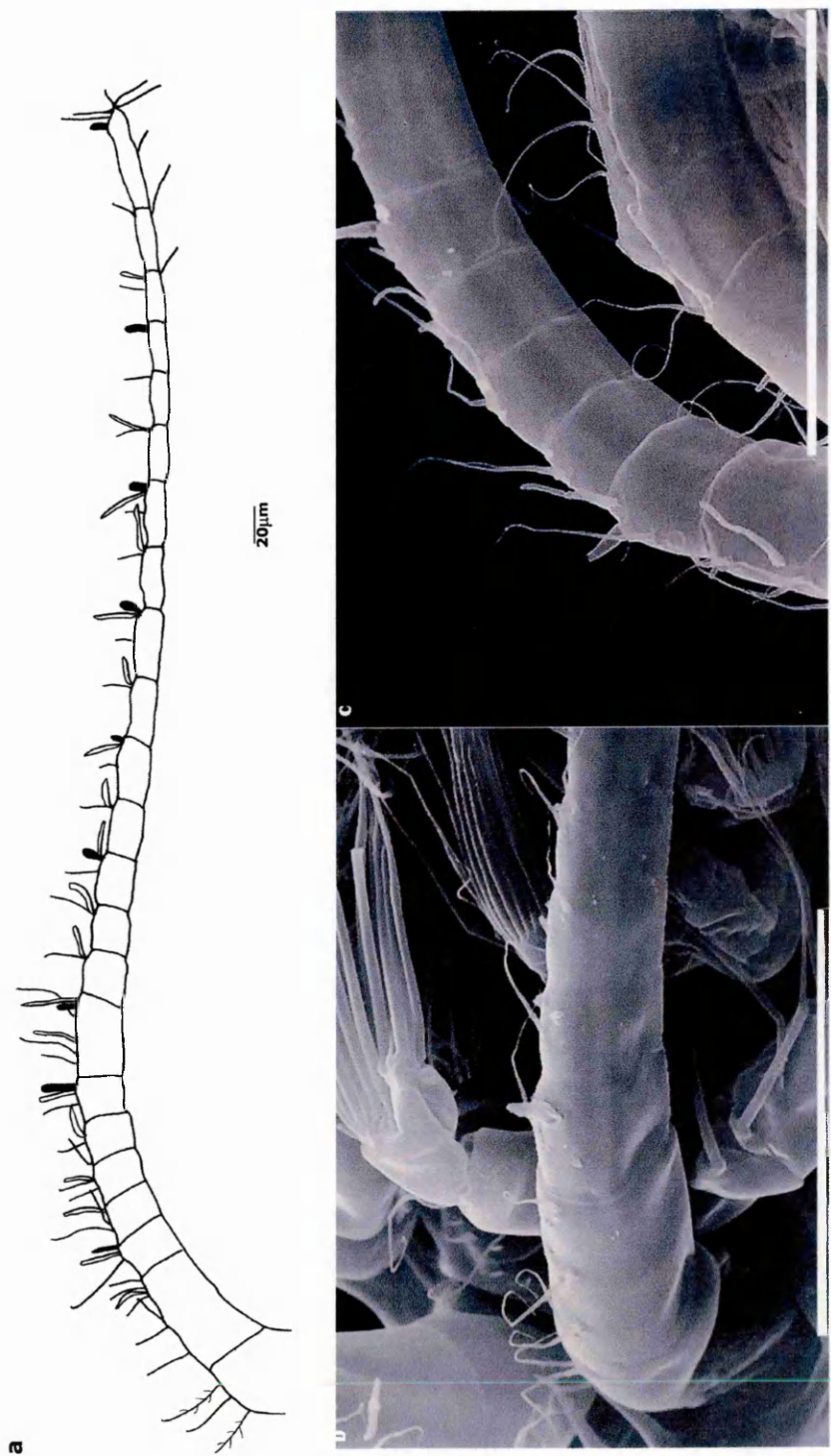


Figure 4.4: alignment of setae and aesthetascs along the margins of the A1 of *C. furcatus* female. a) camera lucida drawing (40X magnification) shows the setation pattern and the disposition of the aesthetascs (cylindrical-like structures) and of the modified setae (in black) along the segments. b & c) SEM pictures show the disposition of the sensory structures along the anterior margin of the basal segments (1 to 7) of the right (b) and left (c) antennules of *C. furcatus* (scale bar=100 μm).

copepods (Fleminger, 1985; Kurbjewit & Buchholz, 1991; Svensen & Tande, 1999; Miller *et al.*, 2005).

All sensory structures are aligned along the anterior margin of the antennules. Only segments 22, 23 and fused segment 24-25 bear one seta each along the posterior margin. The sensory arrangement present on the distal tip (Figure 4.5) comprises: a two simple and one modified seta on the anterior margin, one seta along the posterior edge and a tuft of three outwardly projecting setae on the tip, originating from the same socket.

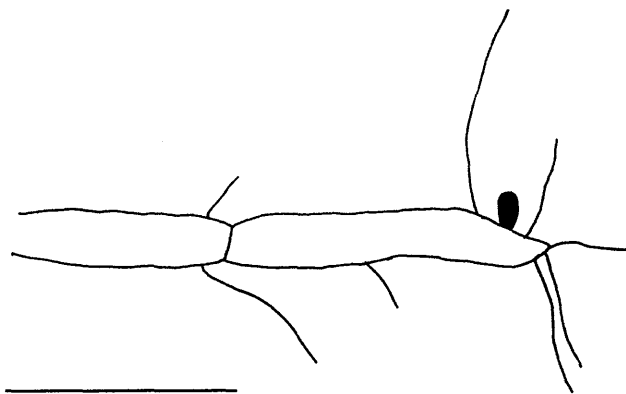
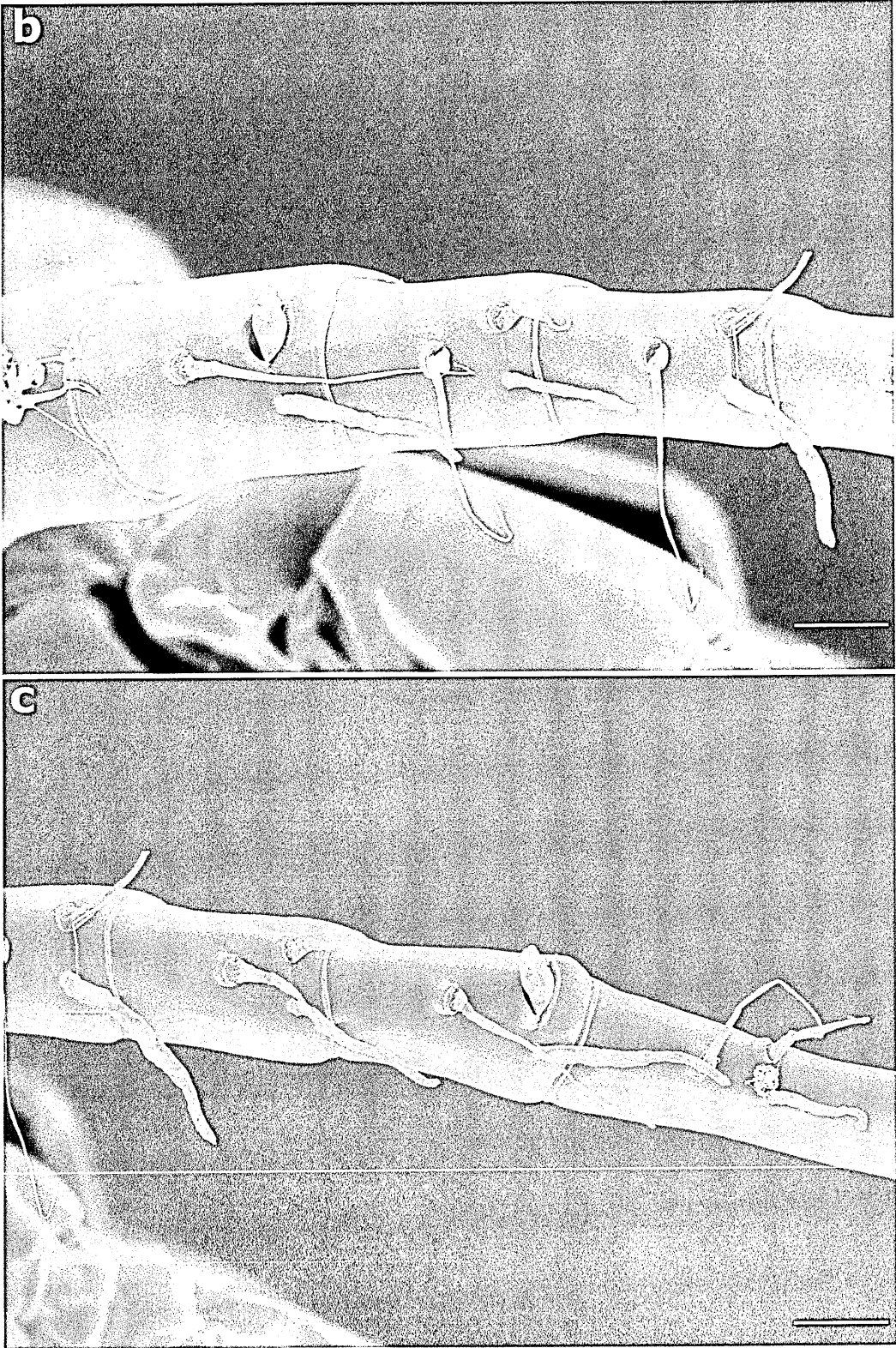
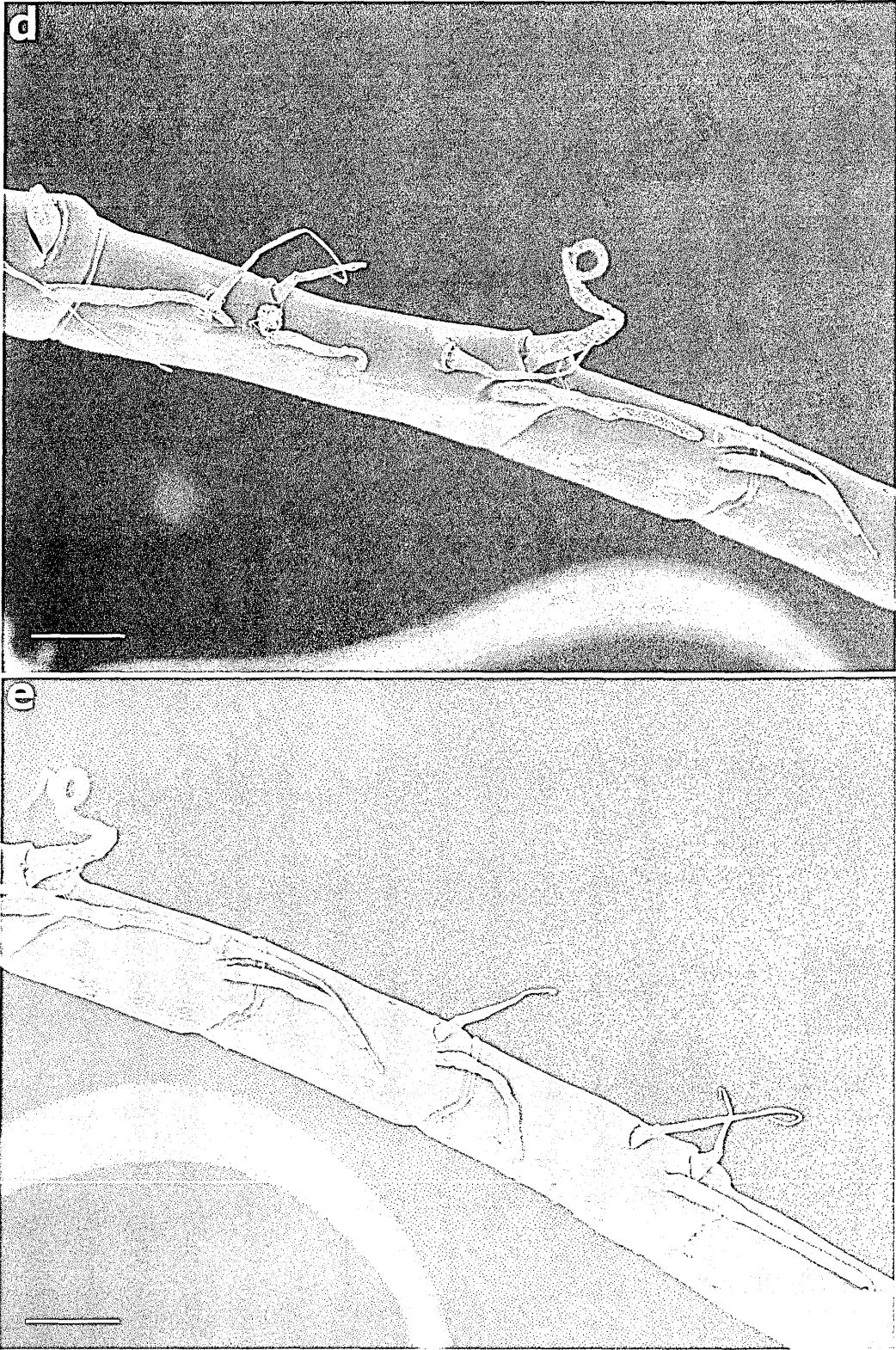


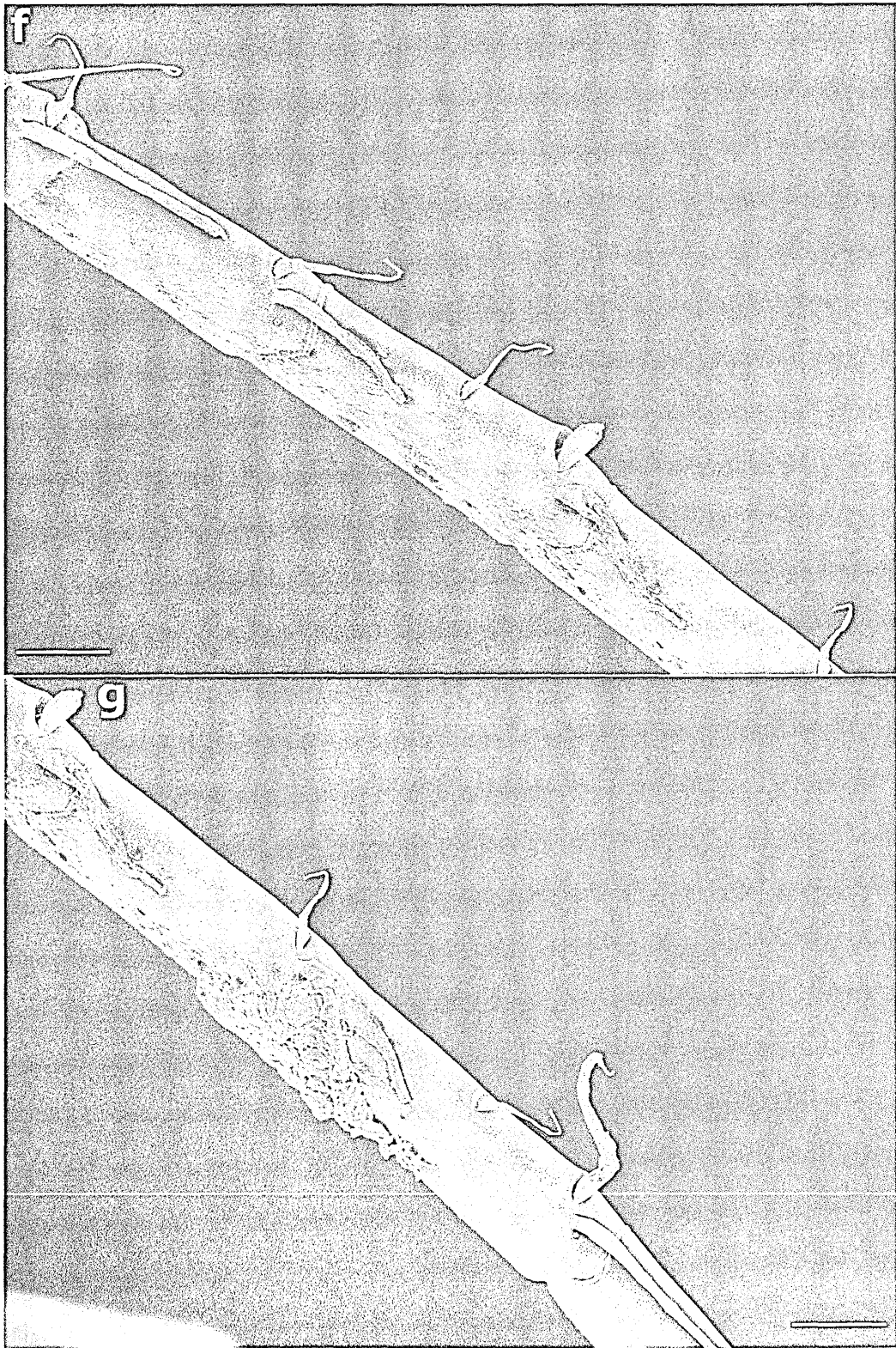
Figure 4.5: details of the distal tip of the first antenna of *C. furcatus* female. Camera lucida drawing (100X magnification) (scale bar=25 μ m) of the last segment, with two simple setae and one modified seta on the anterior margin, a single seta on the posterior edge and a tuft of three setae on the tip of the antennule. It is worth noticing that, unlike other copepods (see text for references), the setae on the tip are not above the longest ones of the antennular system.

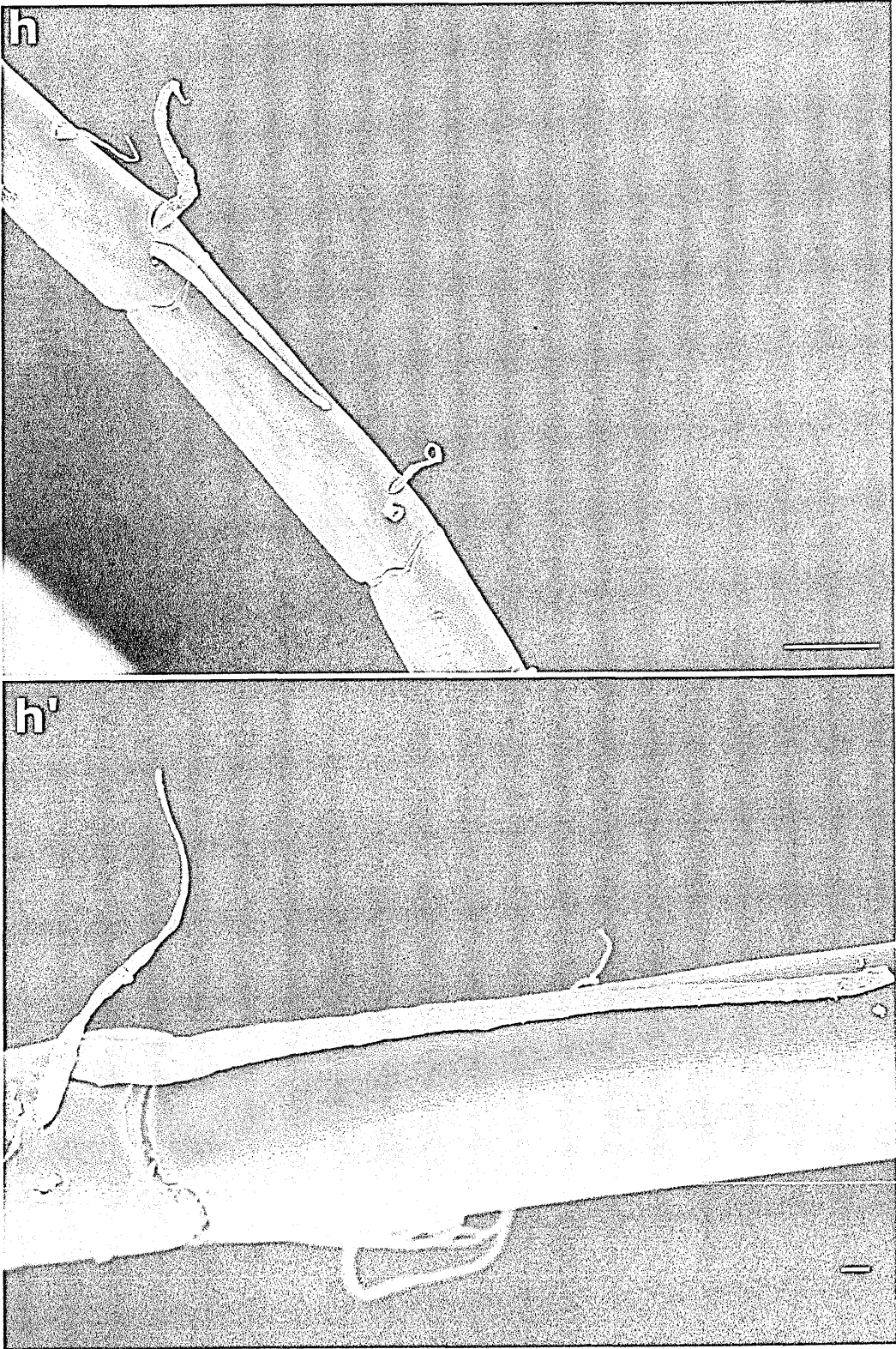
SEM observations provide further details of the typologies of structures present, as well as on the microscale features of the A1s (Figure 4.6). Simple setae (Figure 4.7) are presumed pure mechanoreceptors: they show a smooth external surface and a tapered end; they originate from a circular (approximately 4 μ m in diameter) and symmetrical socket which, coupled with the lack of any one-point innervation at the base of the sensor, provides an indirect evidence of the absence of directional

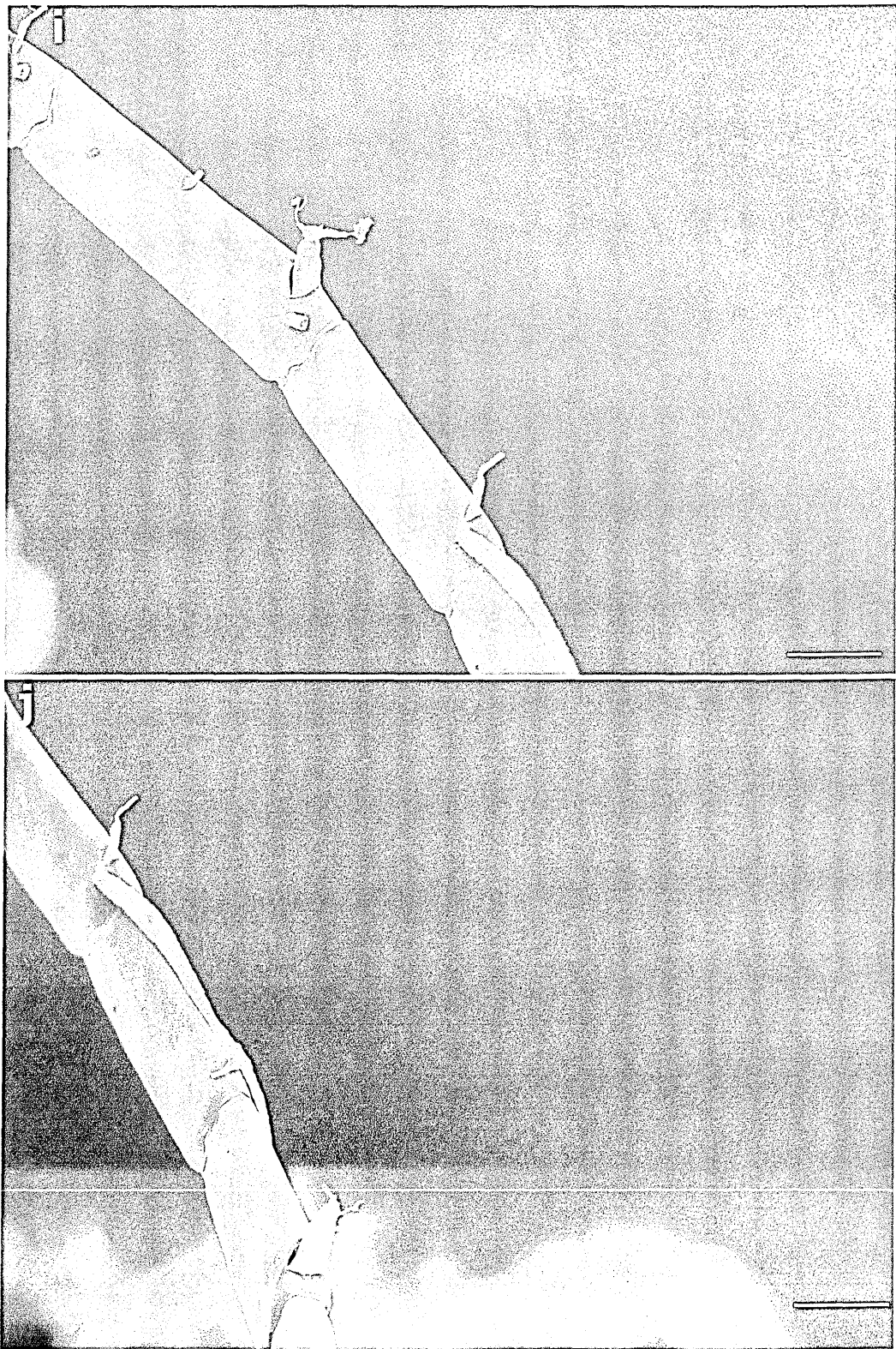












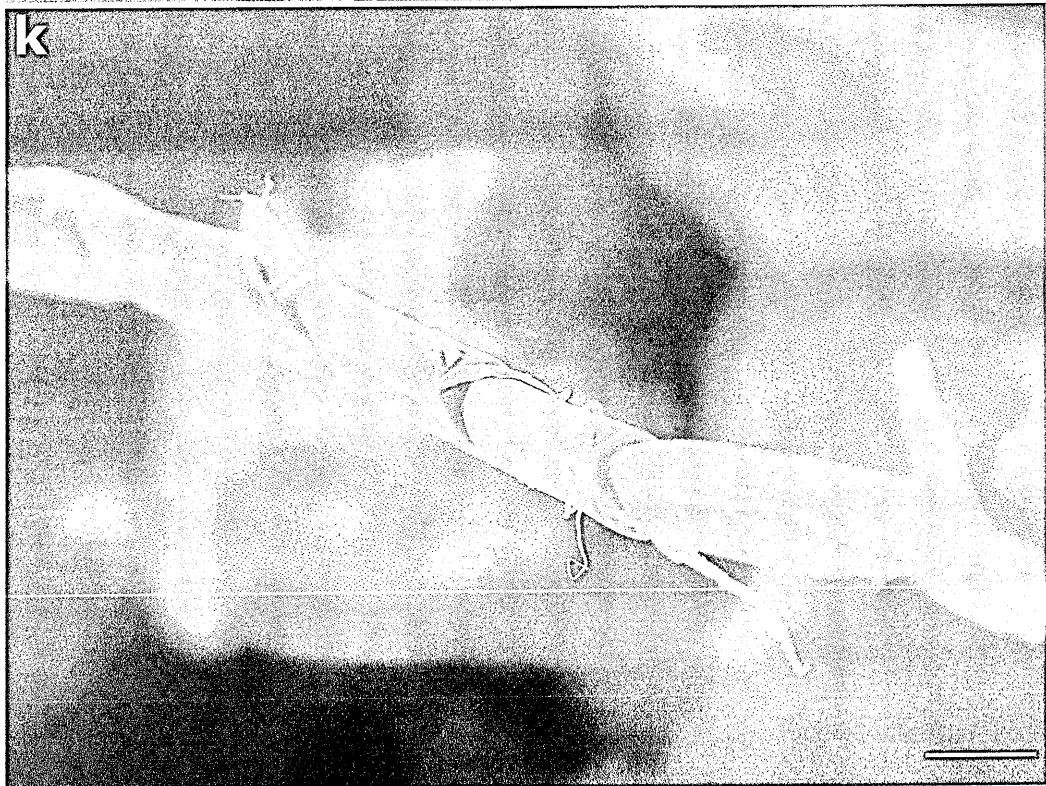
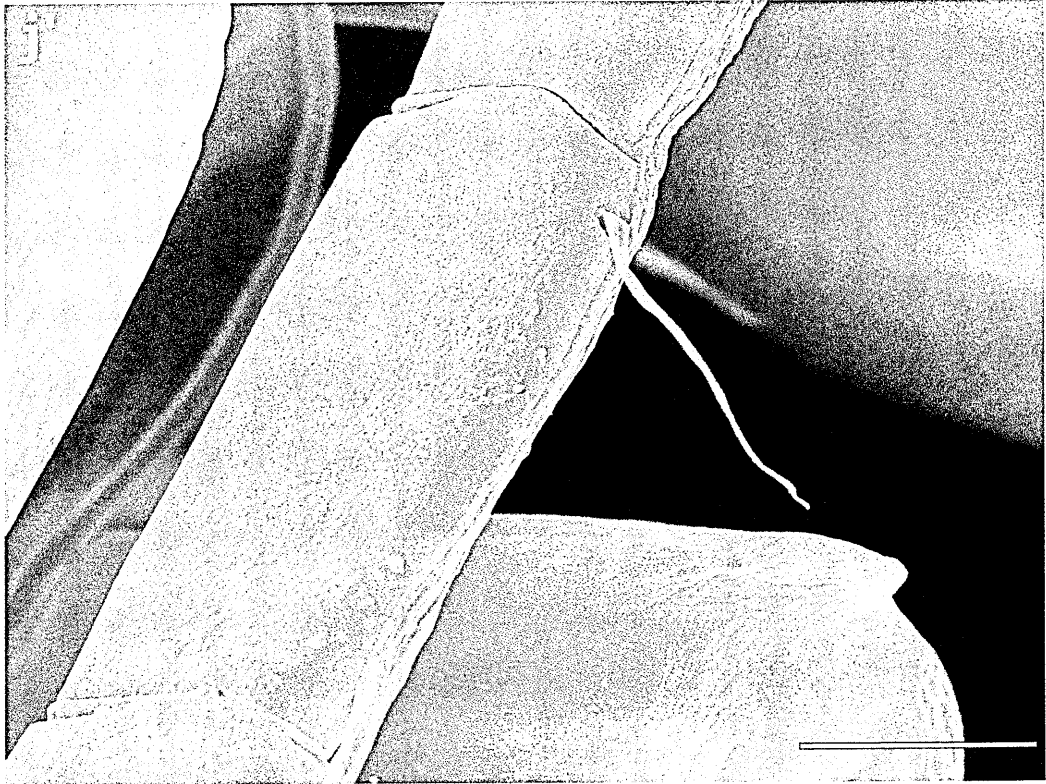




Figure 4.6: SEM mapping of the left antennule of a *C. furcatus* adult female; panels labelled with ' are pictures from different specimens, to show those details not visible in the analogous plate. a) segments 1 and 2 (scale bar=10 μm); a') detail of segment 2, showing the aesthetasc torn in panel a (scale bar=10 μm); b) segments 3 to 5 (scale bar=10 μm); c) segments 6 and 7 (scale bar=10 μm); d) segments 8-9 and 10 (scale bar=10 μm); e) segments 10 to 12 (scale bar=10 μm); f) segments 13 and 14 (scale bar=10 μm); g) segments 15 and 16 (scale bar=10 μm); h) segments 16 and 17 (scale bar=10 μm); h') detail of the two setae brought by segment 17 (scale bar=1 μm); i) segments 18 and 19 (scale bar=10 μm); j) segments 19 to 21 (scale bar=10 μm); j') single seta present on segment 20 (scale bar=10 μm); k) segments 21 to 23 (scale bar=10 μm); l) segment 24-25 (scale bar=10 μm).

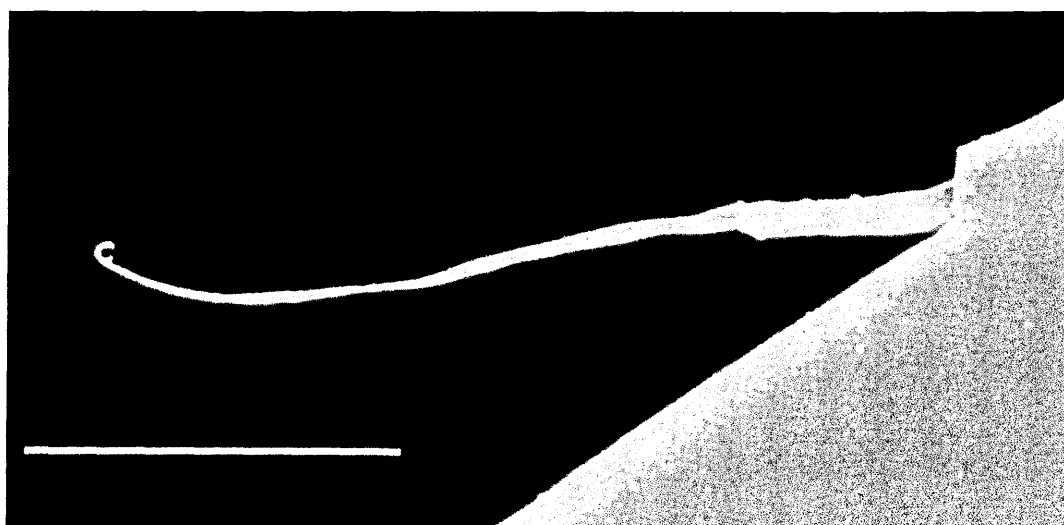


Figure 4.7: detail of a naked seta present on segment 14 of the left A1 of a *C. furcatus* adult female. The picture shows the smooth texture of the sensor, its tapering shape and the characteristic anchoring socket (scale bar=10 μm).

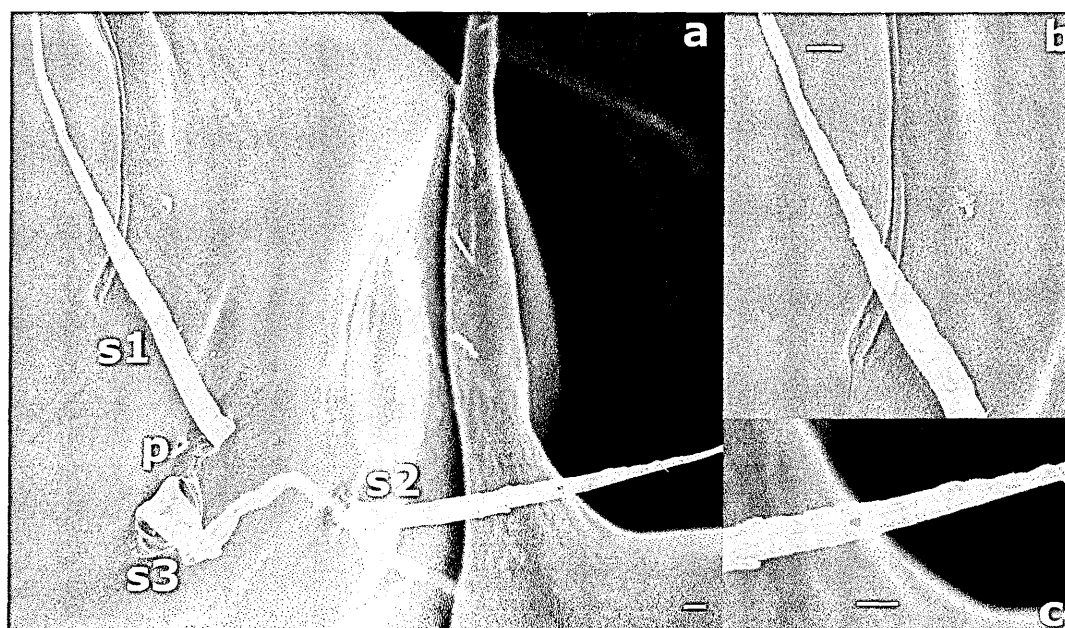


Figure 4.8: segment 1 of the right antennule of *C. furcatus* female. a) disposition of the two serrulate setae (s1 and s2), of the naked seta (s3) and of the pore between s1 and s3 (scale bar=1 μm); b) detail of the setules on s1 (scale bar=1 μm); c) detail of the setules on s2 (scale bar=1 μm).

sensitivity (Bush & Laverack, 1982). Segment 1 (Figure 4.8) is characterised by two peculiar features: it presents the only two setae of the entire A1 equipped with short setules (1-2 μm) (serrulate setae), and a pore between two neighbouring setae. Another pore is sometimes visible close to the socket of the seta on the anterior margin of segment 23, but this character was not discernible in all individuals investigated. No seta bears an apical pore.

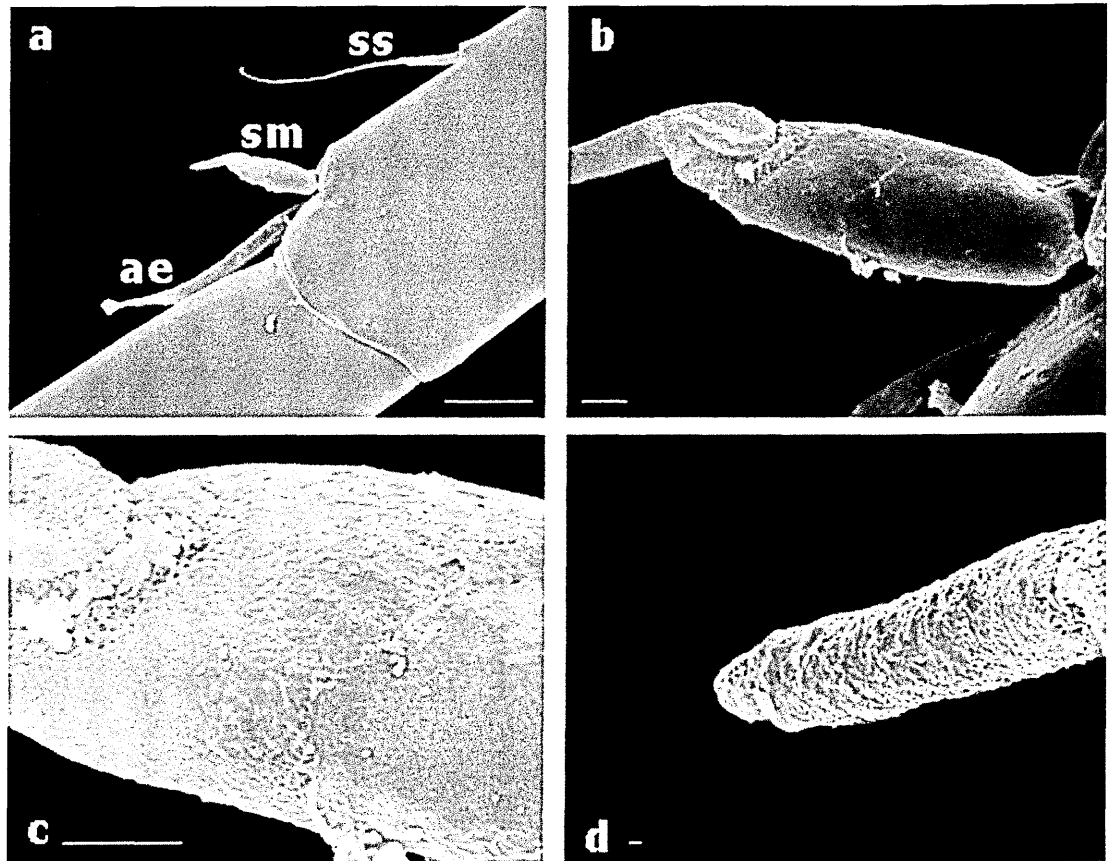


Figure 4.9: SEM pictures of the modified seta on segment 14 of the antennule of *C. furcatus*. a) typical trithek arrangement, with one seta (sm, modified seta) close to the chemoreceptor (ae) and the other (ss, simple seta) on the median part of the segment (scale bar=10 μm). b) morphological features of the modified seta: the neck constriction is clearly recognisable (pointed as *) as well as the tubular shape (scale bar=1 μm). c) detail of the spongy cuticular structure along the body of the sensor (scale bar=1 μm). d) enlargement of the tip of the modified seta (scale bar=100 nm).

Modified setae are presumed to be chemosensitive or dual-function sensors (Figure 4.9). They are characterised by a smooth texture, as in simple setae, whilst the median and distal part of the sensor possess a spongy-like structure typical of copepods aesthetascs. Modified setae are anchored to the antennule by means of a socket larger ($5\text{--}7\text{ }\mu\text{m}$) and deeper than those of simple setae. Their base is characterised by a clearly recognisable neck constriction; the tip does not present any apical pore.

The aesthetascs of *C. furcatus* show the characteristic bulbous shape with a spongy external texture (Figure 4.10a). The root of the sensor protrudes directly from the chitinous surface of the A1 (Figure 4.10b), not being encapsulated in a socket, the base having a diameter of nearly $2\text{ }\mu\text{m}$. Unlike the same receptors found in other

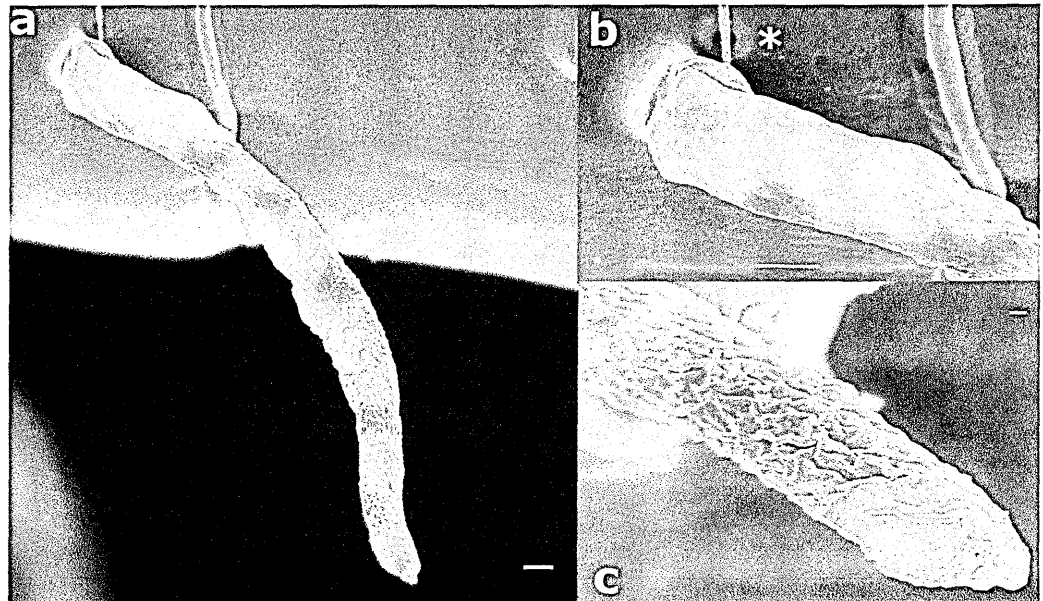


Figure 4.10: aesthetasc on segment 2 of the left antennule of *C. furcatus* female. a) cylindrical shape of the sensillum, with crenulated texture along the entire length of the structure (scale bar= $1\text{ }\mu\text{m}$); b) base of aesthetasc, without anchor socket and neck constriction; it is visible also the circular pore (pointed with *) associated to the base (scale bar= $1\text{ }\mu\text{m}$); c) detail of the tip of the aesthetasc, showing the absence of an apical pore (scale bar= 100 nm).

species (Kurbjeweit & Buchholz, 1991; Bundy & Paffenhöfer, 1993), the aesthetascs of *C. furcatus* do not show the typical neck constriction. The tip does not have any pore (Figure 4.10c). The base of aesthetasc on segment 2 is always accompanied by a pore; this association was occasionally noted also for other modified setae (segments 14, 16 and 19), not representing a common feature in all organisms analysed. All pores reported for the A1 of *C. furcatus* have a circular shape, as described for other calanoids (e.g., Blades & Youngbluth, 1979; Gill, 1986).

The four types sensory structures of *C. furcatus* (simple setae, serrulate setae, modified setae and aesthetascs), identified at the SEM using morphological evidences, were further analysed at TEM to collect details on their internal structure.

Simple setae show the typical characteristics of copepod mechanosensor, i.e. a thick cuticle enveloping a large proliferation of microtubules (Figure 4.11). The cuticle can be recognised by its compact structure, formed by overlapping layers of chitin, whereas microtubules are identified as small black dots. These characteristics are comparable with what already noticed for mechanosensitive setae in other species (e.g., *Pleuromamma xiphias* – Lenz *et al.*, 1996; Weatherby & Lenz, 2000).

Figure 4.12 shows a longitudinal section of one serrulate seta placed on segment 1 of the A1 of *C. furcatus*. In addition to the typical features of simple setae (thick cuticle, presence of microtubules), this structure presents small setules (1-2 μm) without any sort of innervation, indicating that they are just chitinous extensions of the mechanosensor.

Modified setae possess characters typical of simple setae (Figure 4.13): the cuticle is in fact rather thick, and the internal cavity is partly filled with a relatively large number of microtubules. The anchor socket is very large (approximately 7 μm in the case reported in Figure 4.13), and the base of the structure is wide and much larger than that of simple setae. The structure of the cuticle of the sensor is made of consecutive layers of chitin, as reported for *Pleuromamma xiphias* (Lenz *et al.*, 1996, their Figure 2). TEM sections did not allow investigating the terminal part of the

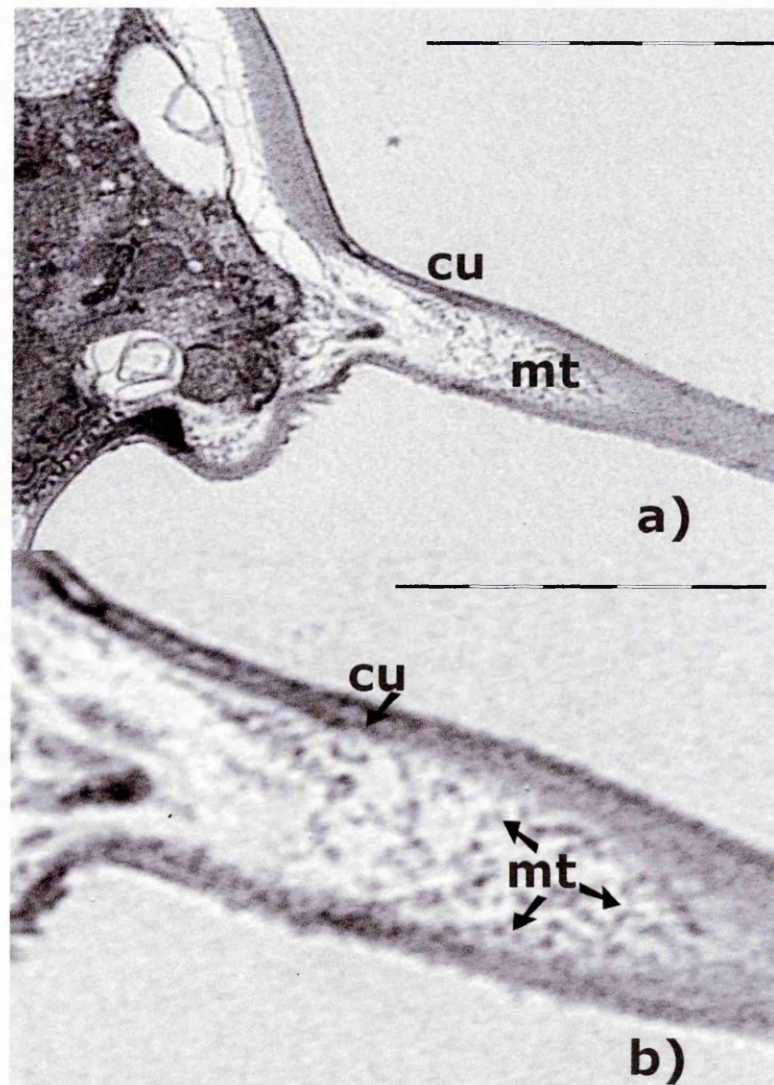


Figure 4.11: longitudinal TEM section of one simple seta on the distal part of the A1 of *C. furcatus*. a) the simple seta is characterised by a thick cuticle (cu) and by the presence of numerous microtubules (mt) (scale bar=5 μm). b) magnification of the base of the setae, showing the massive presence of microtubules (scale bar=2 μm).

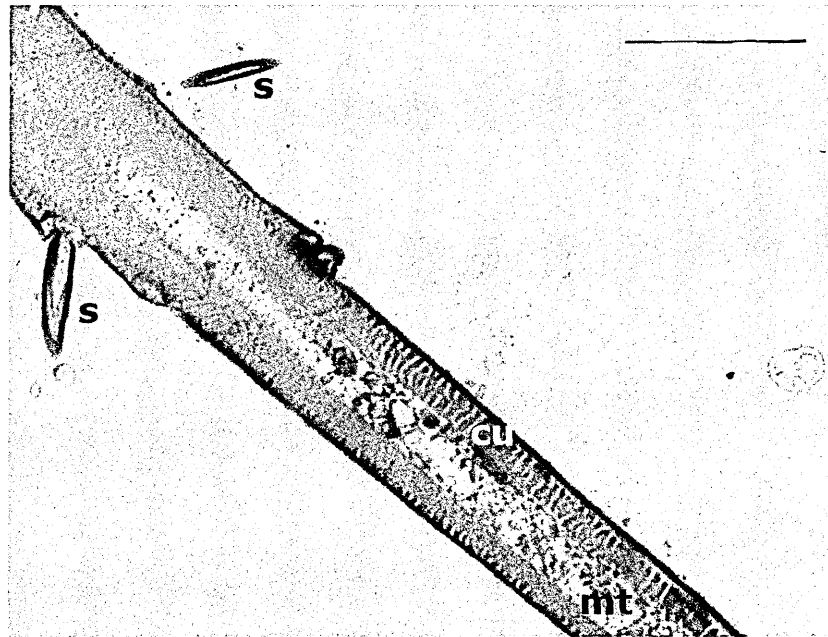


Figure 4.12: longitudinal section of one serrulate seta (segment 1) of *C. furcatus*. The setules (s) are not provided with any nervous termination, but are projections from the chitinous outer surface of the A1. As the simple seta in Figure 4.11, the serrulate seta shows a thick cuticle and numerous microtubules (scale bar=2 μm).

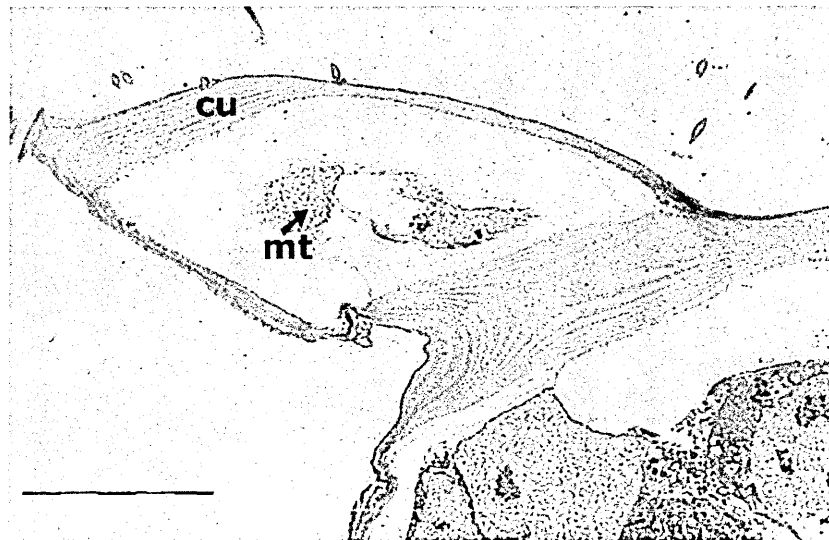


Figure 4.13: longitudinal section of the base of one modified seta, showing typical features of mechanosensitive setae: a thick cuticle enveloping numerous microtubules. It is worth noticing the width of the structure, which is much larger than setae (scale bar=4 μm).

modified seta, where a spongy structure is present (as evident from SEM pictures in Figure 4.9).

The aesthetascs of *C. furcatus* (Figure 4.14) have a very thin and apparently perforated cuticle through which chemical odorants can permeate, coming into contact with the nervous structures of the sensor. Another marked difference compared to seta lies in the lack of cluster of microtubules, a distinguishing feature of copepod chemoreceptors. The dark circular profiles evident in the TEM section may likely be the ends of forked dendrites (T. Weatherby Carvalho, Biological Electron Microscope Facility-University of Hawaii at Manoa, *pers. comm.*), whose presence further supports the chemosensitive function of this structure (Lenz *et al.*, 1996).

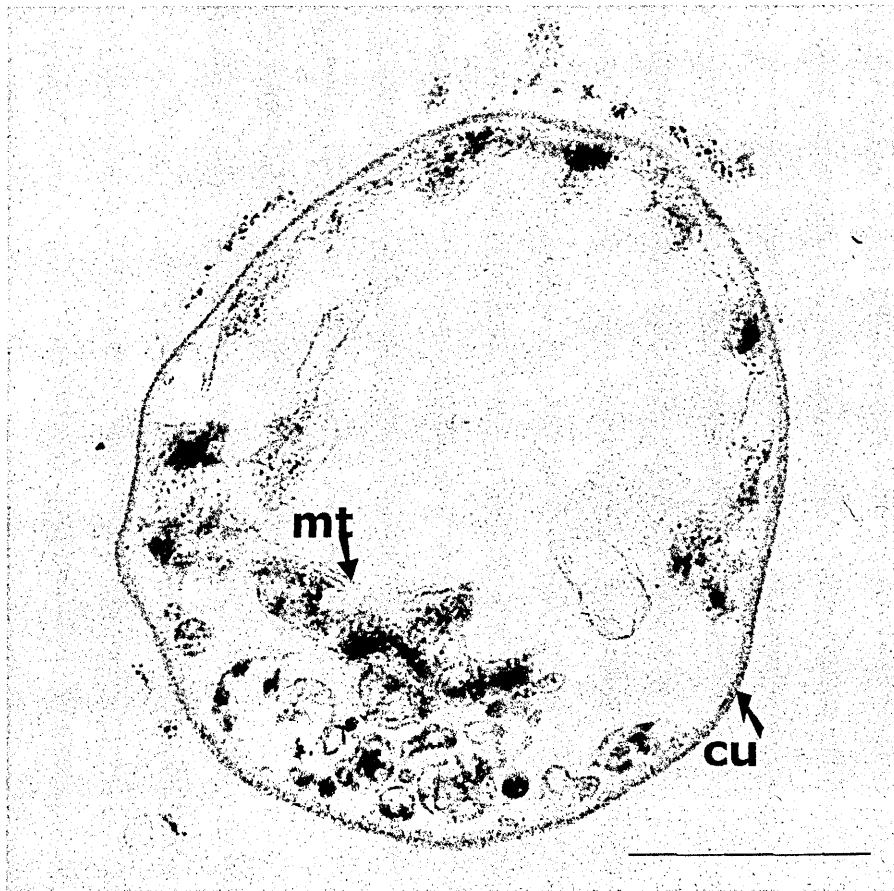
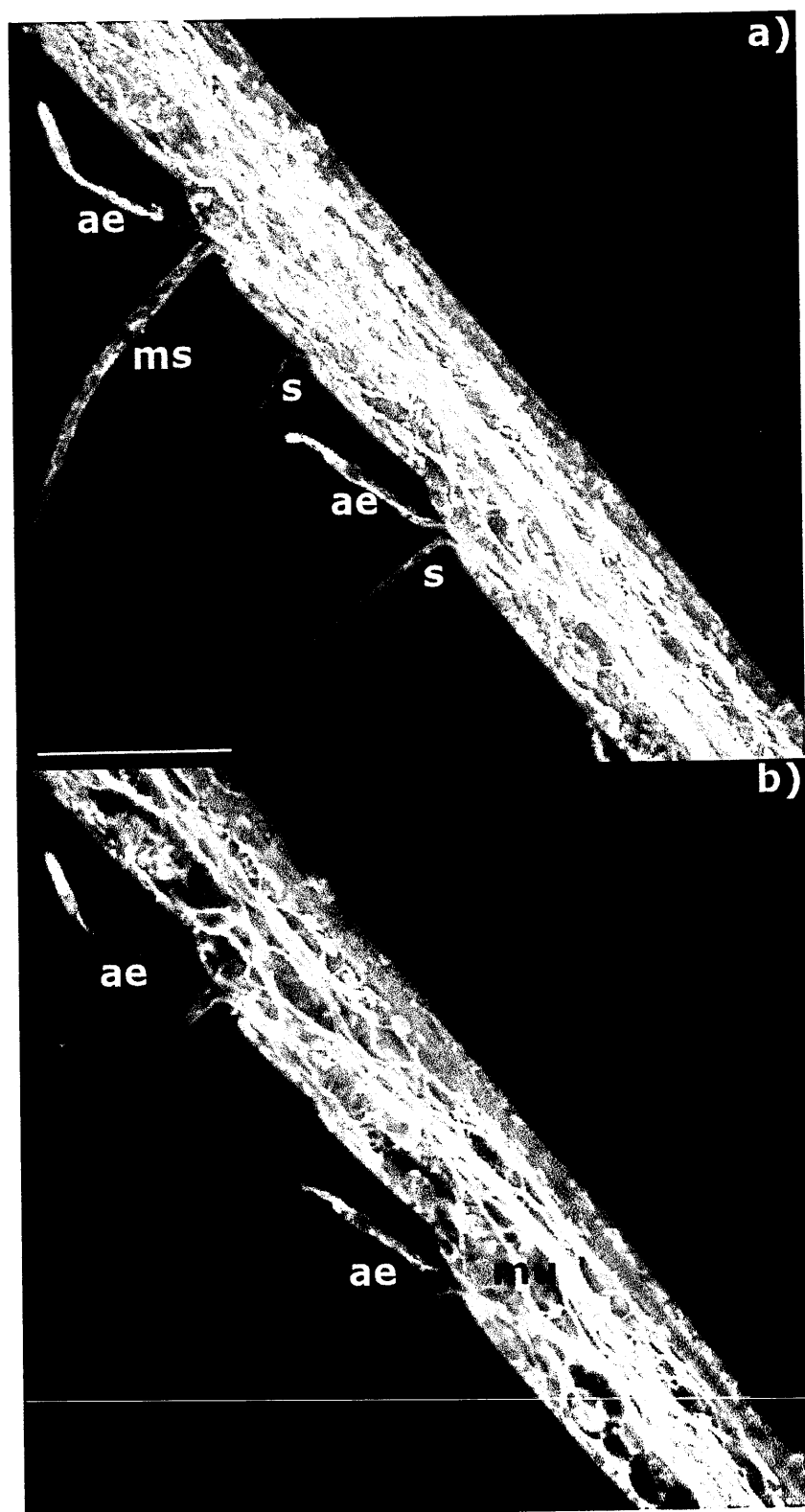


Figure 4.14: TEM cross section of one aesthetasc located on the distal tip of the A1 of *C. furcatus*. The chemosensitive function of this structure is underlined by the presence of a thin cuticle and by the presence of few microtubules (scale bar=1 μm).

Further details on the functioning of the different sensors are achieved by the investigation by means of LSCM. Dye uptake by the structures analysed can in fact be read as an indirect evidence of their chemical sensitivity (Lenz *et al.*, 1996). Compiled confocal images (Figure 4.15a) show that aesthetascs brightly fluoresce, that modified setae are weakly and not-uniformly fluorescent whereas simple setae do not fluoresce at all. These different results are a consequence of the specific morphological and structural peculiarities of each sensor, as described through SEM and TEM, and can be better appreciated looking at single optical sections. Aesthetascs fluoresce as a consequence of the absorption of DiI through their spongy cuticle (Figure 4.15b); the assimilation of the dye occurs rather uniformly along the entire length of the sensor. Muscle bands are clearly recognisable as well (Figure 4.15b).

Simple and modified setae show different responses (Figure 4.15c). The former do not fluoresce at all: they do not absorb DiI owing to their thick chitinous cuticle, which is almost impermeable to chemical molecules. The response of modified setae is intermediate between that of aesthetascs and that of simple setae. These structures in fact show some spots of fluorescence (labelled with * in Figure 4.15c) towards the median/distal part, in correspondence of the spongy texture noticed through SEM (see Figure 4.9). By contrast, no fluorescence is noticed at the base of the modified seta, where a thick cuticle is reported (Figure 4.13). Owing to the absence of an apical pore, the only means of dye absorption is represented by the spongy cuticle of the sensor.

The complementation of SEM, TEM and LSCM analyses provide a complete scheme of the distribution and typology of the antennular sensors of *C. furcatus*. Simple setae are pure mechanoreceptors: their external smooth structure is made of a thick chitinous layer, and they are provided with a large number of microtubules. The lack in DiI absorption further supports the pure mechanosensitive function of this structure. The setules brought by serrulate setae are pure chitinous structures, without any neuronal tissue in them.



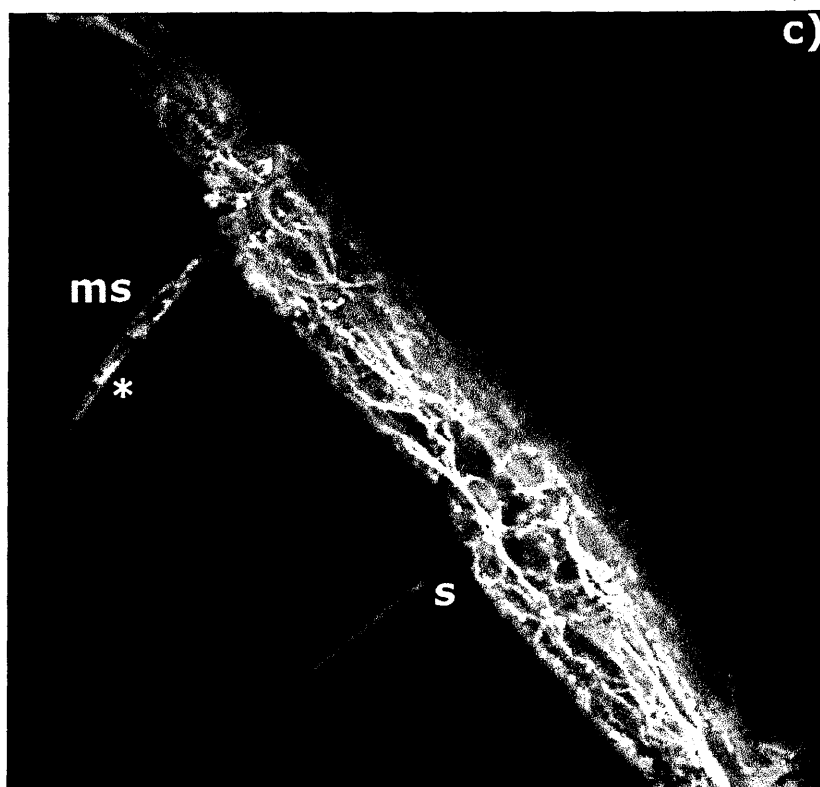


Figure 4.15: LSCM pictures of segments 15 and 16 of the A1 of *C. furcatus*. a) compiled image of the optical sections taken; this stacked imaged shows brightly fluorescent aesthetascs (ae), modified setae (ms) with bare fluorescense and not-fluorescent simple setae (s). b) optical section (optical thickness=1 μm) showing fluorescent aesthetascs (ae) as a result of an uniform DiI absorption throughout the sensor; in the section are also evident the muscle bands (mu) running trough the A1. c) optical section (optical thickness=1 μm) highlighting the localised spot of fluorescence (*) along the modified seta (ms); by contrast, the simple seta (s) does not show any fluorescence. Scale bar=20 μm for all panels.

Modified setae possess morphological characteristics intermediate between simple setae and aesthetascs; their base is very large, is composed of several superimposing chitin layers, and possess numerous microtubule; on the other hand, their tip present a spongy-like structure indicative of a presumed chemosensitive function. The dual functioning of this structure is demonstrated by the localised absorption of DiI in

confined regions of the distal part, in correspondence of the spongy structure observed at SEM.

The aesthetascs of *C. furcatus* show the typical spongy external texture typical of copepod chemosensors. TEM sections highlight the presence of few microtubules and of a thin cuticle, and in LSCM images aesthetascs can be easily recognised owing to their bright fluorescence, as a result of an efficient penetration of the dye through the permeable cuticle of the sensor.

Table II provides a map of the pattern of distribution of the sensory array along the antennule of *C. furcatus* female, obtained from the comparative observations using CLDs, SEM pictures and LSCM reconstructions.

Table II: summary of the disposition of the sensory structures along the first antennae of *C. furcatus* female, obtained by the comparative analysis of CLDs, SEM pictures and LSCM reconstructions.

Ancestral Segmentation	Actual Segmentation	Simple Setae	Modified Setae	Aesthetascs
I	1	1+2s	0	0
II-IV	2	6	0	1
V	3	1	1	1
VI	4	2	0	1
VII	5	2	0	1
VIII	6	2	0	1
IX	7	1	1	1
X-XI	8-9	3	1	2
XII	10	1	0	1
XIII	11	1	0	1
XIV	12	1	1	1
XV	13	1	0	1
XVI	14	1	1	1
XVII	15	1	0	1
XVIII	16	1	1	1
XIX	17	1	0	1
XX	18	1	1	1
XXI	19	1	0	1
XXII	20	1	0	0
XXIII	21	0	1	0
XXIV	22	1+1*	0	1
XXV	23	1+1*	0	0
XXVI-XXVIII	24-25	2+1*+3**	1	0

*: on the posterior margin of the antennule

**: on the distal tip, grouped in a tuft

s: serrulate seta

4.4.2 Neurophysiology

The NITs recorded for the three sectors of the antennule of *C. furcatus* showed peculiar traits and characteristics. In the absence of mechanical stimuli, NIT recordings showed an almost flat baseline (Figure 4.15); the small low-amplitude spikes present can be assumed to be a noise artefact or, alternatively, as spontaneous low-intensity activity.

When the stimulus was applied, the situation drastically changed. The basal area (Figure 4.15) showed the most intense neurophysiological activity: the detection of the mechanical cue is represented by the sharp spikes in NIT recordings, well above the physiologic baseline, attaining maximum values up to 60,000 pA ($39,257.4 \pm 13,768.4$ pA; mean \pm standard deviation). Latency times, representing the time lag between the beginning of the stimulus and the onset of the neurophysiological response, were on average ~ 90 ms.

The median area (Figure 4.15) showed less pronounced responses. Spikes were still present, but they achieved values of just $\sim 10,000$ pA ($11,487.5 \pm 5,886.4$ pA), i.e. nearly 3 times less than those recorded for the basal sector. Latency times were similar to those reported for the first segments, with average values of about 80 ms.

The distal sector showed reduced reactions to the mechanical stimulus applied (Figure 4.15). NIT recordings were almost flat, with low-amplitude spikes ($2,796.2 \pm 275.6$ pA) occurring nearly 85 ms after the onset of the trigger.

To verify that the spikes recorded were effectively associated with the neurophysiological responses of the specimen analysed, the basal area (where responses were most intense) was stimulated in presence of TTX. The neurotoxin took approximately 15 min (at the concentration utilised in the experiments) before blocking the ion channels. At the beginning of the experiment (Figure 4.16a), NIT recordings showed the same pattern observed for the normal condition (i.e. without the toxin). When TTX became effective (Figure 4.16b and c), however, the spikes and the associated train of impulses reduced in amplitude until disappearing completely. The small spikes displayed were likely due to a mechanical artefact connected to the

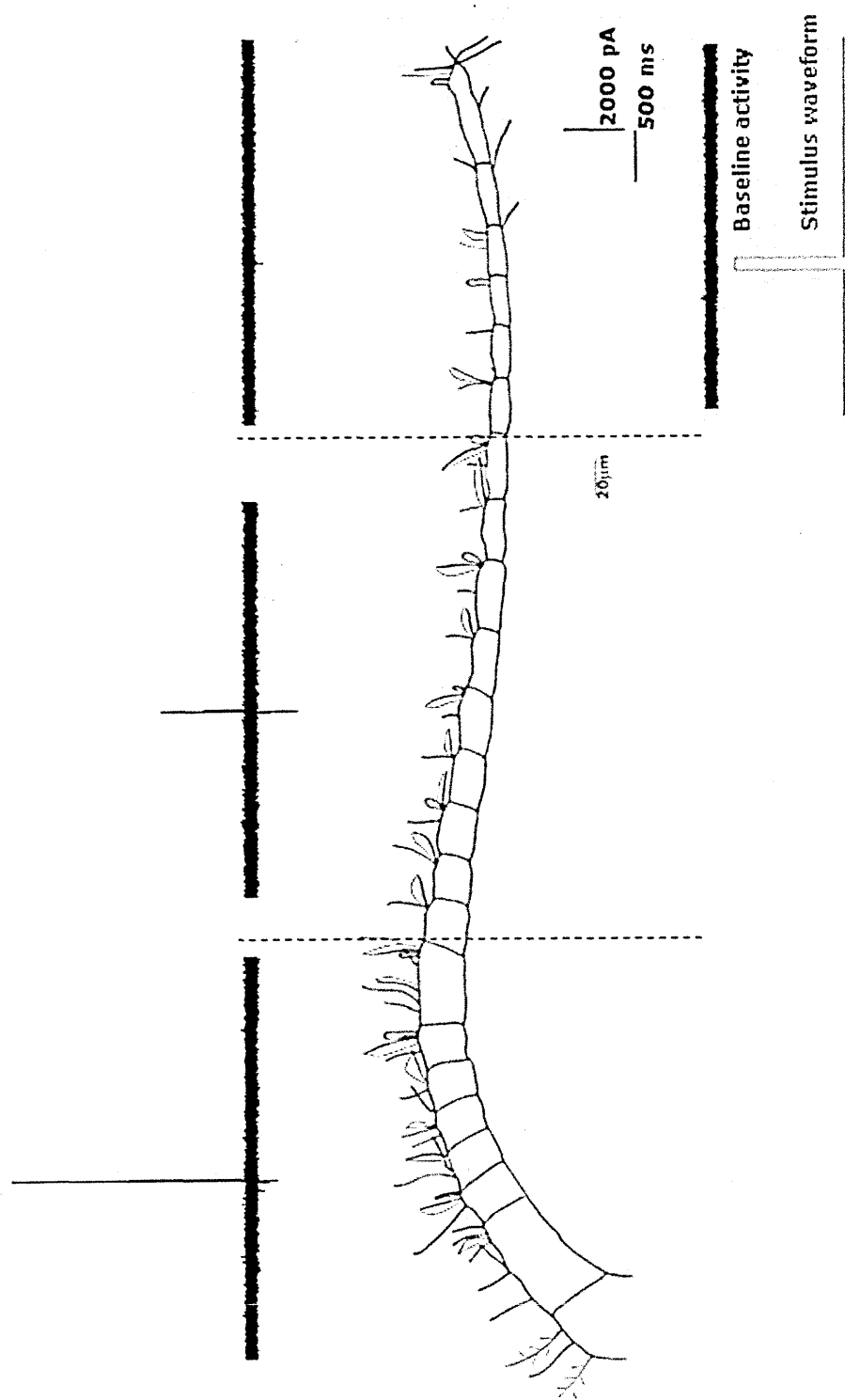


Figure 4.15: NIT recordings for each sector of the A1 of a *C. furcatus* adult female. The strongest responses were associated with the basal sector, whereas the distal segments showed minimal effects; the median sector had an intermediate response.

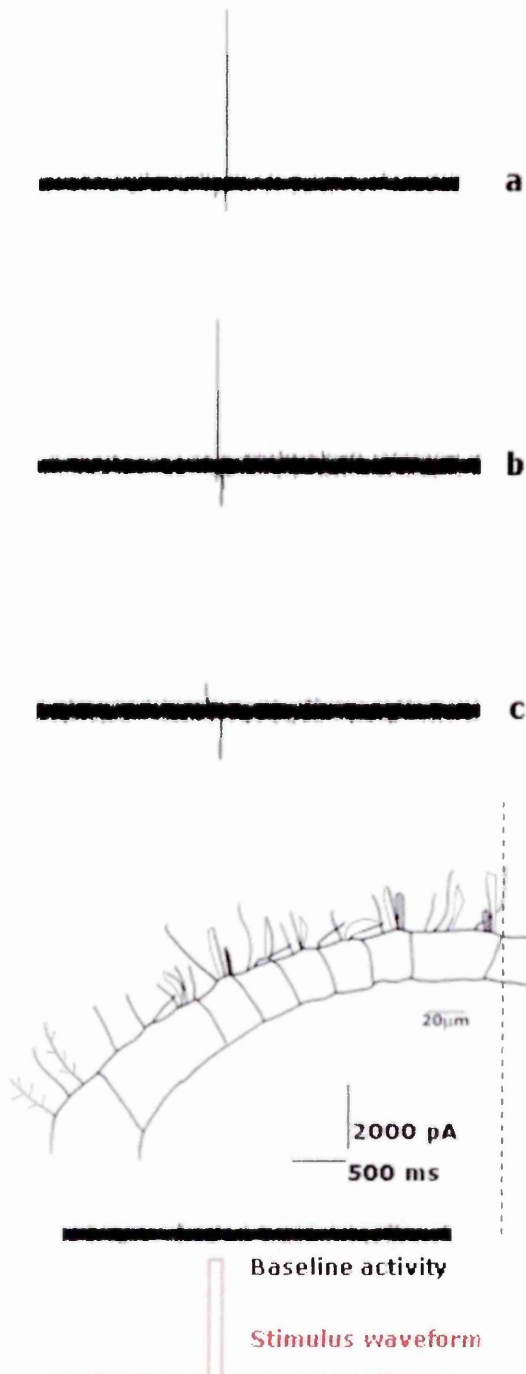


Figure 4.16: TNI recordings of the A1 of *C. furcatus* female under the effect of TTX. At the beginning of the experiment, the basal sector showed its typical responses (a); after about 10 minutes from TTX addition, the A1 reduced its neurophysiological fitness (b), until complete blockage of ion channels was obtained after nearly 15 minutes (c). The stimulus waveform signal is sketched in panel d.

stimulus itself; they were in fact constantly preserved during the recordings, even after one hour from the beginning of the effect of the neurotoxin, and were perfectly synchronised with the onset and the end of the trigger. TTX experiments therefore validated the reliability of the TNI recordings obtained in the previous trials, indicating that the spikes observed were due only to the neuronal response of the sensory structures.

4.5 Discussion

The antennules of *C. furcatus* adult female are equipped with short simple setae characterised by circular and symmetrical sockets, indicating that this small calanoid utilises its mechanoreceptors to sense directionally free mechanical cues. Setae are more abundant over segments 1 to 8-9, and become sparser in the median and distal sectors of the A1, according to the scheme proposed by Lenz & Yen (1993). In addition, segment 1 bears two serrulate setae: these setules increase the effective surface of the first antenna (Laverack, 1968), improving the sensitivity to mechanical stimuli.

The A1s of *C. furcatus* also bear numerous aesthetascs and presumed chemosensitive setae, suggesting that the copepod depends on chemical cues as well to detect its prey and to acquire information from the surrounding environment.

The sensory structures (both setae and aesthetascs) are aligned along the anterior margin of the antennule, as in other actively swimming copepods (e.g., *Temora longicornis*: Gill, 1985; *Euchaeta norvegica*: Yen & Nicoll, 1990; *Pleuromamma xiphias*: Lenz & Yen, 1993, Weatherby *et al.*, 1994; *Euchaeta rimana*: Lenz & Yen, 1993, Doall *et al.*, 2002, Fields & Yen, 2002; *Diaptomus sicilis*: Bundy *et al.*, 1998; *Centropages typicus*, *Temora stylifera*, *Paracalanus parvus* and *Pleuromamma gracilis*: *pers. obs.*), In all the above mentioned species, only the last one to three segments have a seta also along the posterior margin.

The arrangement and typology of sensory structures provide useful information to understand the adaptations of the species to its natural environment. *C. furcatus* appears to be dependent on both mechanical and chemical cues. The presence of setae shorter than the ones present on the A1s of similar-sized cruising species (e.g., *Paracalanus parvus*, *Paracalanus aculeatus*) suggests that *C. furcatus* may not rely on mechanoreception as extensively as small calanoids. In addition, simple setae are more abundant on those segments corresponding to the capture area of the copepod. This evidence may imply a critical role of mechanical signals in the localisation of prey items just before their capture. The presence of chemoreceptors (aesthetascs and modified setae) along the entire A1, and more particularly on the antennular segments outside the capture area, hints at an efficient utilisation of chemical cues to localise potential food item far away from the capture area.

These observed sensory structures seem to be well suited for the characteristic swimming behaviour of *C. furcatus*. Short setae are more compatible with a fast and continuous motion, whereas longer setae are more fitted for the detection of mechanical cues in smoothly-cruising or sinking copepods. Chemoreception, on the other hand, is enhanced by fast swimming. Detection of chemical cues occurs when an odorant molecule comes into contact with the surface an appropriate detector. By swimming continuously and at high speed, the copepod increases the net flux of water through the sensors, thus increasing the probability of detecting a suitable cue. In addition, the fast motion reduces the boundary layer around the sensors, with a further enhancement of the flux. The A1 sensors of *C. furcatus* can therefore be interpreted as an optimal arrangement for acquiring information from the surrounding environment given this specific swimming behaviour.

Several authors have focused on the study of the distal tip of the antennules. Direct stimulation (Gill, 1985) and electrophysiological measurements (Gassie *et al.*, 1993; Lenz & Yen, 1993) indicate that this area is the most sensitive of the entire A1, playing a crucial role in detecting stimuli from the far field (especially from potential predators). The last segment, in fact, generally has the longest setae (or among the

longer ones), often grouped in a tuft (Gill, 1985; Yen & Nicoll, 1990; Yen *et al.*, 1992; Lenz & Yen, 1993; Weatherby *et al.*, 1994; Fields *et al.*, 2002; Fields & Weissburg, 2004). However, the pattern displayed by *C. furcatus* shows some differences with the above listed citations. In this case, in fact, the distal tip bears one modified seta and two simple setae along the anterior margin, one seta along the posterior edge and a tuft of three setae originating from the same socket on the tip. In *C. furcatus*, distal setae are not among the longest ones of the A1, suggesting that their sensitivity to mechanical cues might be reduced with respect to that of longer setae present on the basal sector of the antennule. On these premises, a question arises: how can *C. furcatus* detect at a distance a potential predator and escape from it? Data on direct stimulation of the distal tip with a predator-mimicking stimulus are missing. However, by describing convoluted tracks, with characteristics resembling those of a random process (as discussed in Chapter 2), *C. furcatus* may hide itself by not providing easily distinguishable mechanical cues to its potential predators. In this way, the behaviour of the copepod might compensate for the apparent reduced sensibility of the distal tip.

Following Paffenhöfer (1998), it is possible to relate the observed A1 structure with the swimming activity of the copepod. The forward projecting sensors result in a sensory horizon predominantly located anteriorly to the head of the animal. The antennular structures acquire information from a portion of fluid not yet disturbed by the motion of the copepod itself (Yen, 2000), thus providing the animal with fundamental cues on which to base its behaviour. This appears to be a typical character in all actively swimming copepods (see above reported citations), which might be more prone to collect information from a portion of fluid which has not yet been explored. In such case, the presence of a well developed distal tip may result in a great enlargement of the sensory horizon available. A completely different scenario arises for a passive sinker such as *Oithona plumifera* (Paffenhöfer & Mazzocchi, 2002), whose sensilla are displaced in a three-dimensional arrangement (Paffenhöfer, 1998) in order to maximise the extension of the capture area.

These strictly morphological details suggest that the sensory performance of the mechanoreceptors of *C. furcatus* is drastically reduced compared to that of other cruising copepods, especially at the level of the distal tip which bears a structurally simple system of sensors. More details on the neurophysiological responses of the copepods come from the electrophysiological experiments performed during this thesis, which represent the first attempt at recording NIT in a small calanoid.

The stimulation of the antennules of *C. furcatus* using a prey-mimicking cue provides details of paramount importance on the sensory capabilities of the copepod. In the presence of the mechanical signal, NIT recordings display sharp peaks, clear indication of the neurophysiological response of the setae on copepod's A1s conveying the peripherically detected signal towards the central nervous system (Yen *et al.*, 1992). This response is in agreement with what already observed for other species (Yen *et al.*, 1992; Lenz & Yen, 1993; Fields & Weissburg, 2004), for which similar spikes have been reported. The experiment performed using TTX confirms that spikes recorded are biologically derived, being due only to the perception of the mechanical cue.

The three sectors of the antennule show marked differences in their sensitivity. On stimulation, the basal area responds with the largest spikes, whereas for the distal sector the smallest reactions are reported; an intermediate reaction is instead present on the median area of the A1. These responses appear directly associated with the setal arrangement of the antennule. The most intense reactions are observed for those segments bearing a higher number of setae (basal sector), while the reduced spikes recorded for the distal segments are associated with a poorly developed sensory array. In addition, the presence of serrulate setae on the first segment might enhance the perception of mechanical stimuli in the basal sector. The sensitivity can consequently be directly related to the arrangement of sensilla present along the A1.

The reduced response of the distal tip represents a rather unique feature. As already mentioned above, this part of the antennule has been reported as the most sensitive for several copepods, displaying giant spikes on stimulation (Lenz & Yen,

1993; Hartline *et al.*, 1996;); instead, in *C. furcatus* the last segments seem not to be efficient in detecting prey-mimicking disturbances. This result might be due to the reduced number of mechanosensory setae along the distal sector of the A1, which are less abundant and shorter than in the other sectors; such reduced system of setae represents a peculiar trait of *C. furcatus* compared to other cruising copepods (Gill, 1985; Yen & Nicoll, 1990; Yen *et al.*, 1992; Lenz & Yen, 1993; Weatherby *et al.*, 1994; Fields *et al.*, 2002; Fields & Weissburg, 2004). In addition, the reduced intensity of the neurophysiological responses to a prey-mimicking stimulus might be also read in terms of predatory strategy. As discussed in Chapter 3, *C. furcatus* collects only those prey which are located almost frontally to its head; in addition, the analysis of video sequences show that the copepod does not modify its swimming behaviour when preying upon a food particle. As a consequence, the lack in sharp responses in association with the distal part of the A1 can be read as an optimisation of the sensitivity of only those sectors of the A1 which are in correspondence of the capture area.

Even though Clausocalanidae bear myelin sheaths (Lenz *et al.*, 2000; Weatherby *et al.*, 2000), observed latency times are higher than the ones reported for other copepods (Yen *et al.*, 1992; Lenz *et al.*, 2000; Fields & Weissburg, 2004). Such discrepancy can be justified by considering the differences in the protocols utilised. The experiments carried out in this work were aimed at mimicking a prey, while other authors focused on the reaction responses of copepods to predator-mimicking cues by utilising stronger signals (e.g., Lenz & Yen, 1993; Lenz *et al.*, 2000; Fields *et al.*, 2002).

Based on available literature on this topic, the electrophysiological experiments discussed in this thesis present a novel contribution. These results represent the first data on the NITs and on the neurophysiological fitness of *C. furcatus*. Moreover they show the different responses of the three sectors of the A1 to a prey-mimicking stimulus, while for other copepods the entire antennule or only its distal part were considered (e.g., Yen *et al.*, 1992; Lenz & Yen, 1993; Lenz *et al.*, 2000; Fields *et al.*,

2002; Fields & Weissburg, 2004). Gill (1985) mapped the sensitivity of the setae along the A1 of *Temora longicornis*, looking at behavioural reactions associated with stimulation but without recording any NITs. In addition, compared with other organisms investigated (see above citations), *C. furcatus* represents the smallest copepod for which neurophysiological activity has been registered so far.

From the results presented here, it is clear that the sensory array and the neurophysiological fitness of *C. furcatus* are closely related. By merging the results of these two different perspectives it is possible to get a comprehensive picture of the mechanisms by which the copepod interacts with its prey, gaining information of primary importance for its survival. Compared to other actively swimming species, *C. furcatus* presents peculiarities and distinctive traits that have never been reported before. This adds to the singularity of behaviour already noticed about the swimming and feeding mechanism, appearing intimately connected with them.

Chapter 5

**The behaviour of
Clausocalanus furcatus:
towards a comprehensive description**

5.1 An integrated account of the behaviour of *C. furcatus*

In this thesis, for the first time the behaviour of *C. furcatus* adult females has been investigated utilising different approaches. For the first time, details on swimming, feeding and neurophysiologic traits are integrated to depict a comprehensive scenario of the interactions occurring at the individual scale between the copepod and its food environment. In addition, for the first time the trajectories of a copepod are characterised in terms of their Lagrangian properties; in the field of zooplankton behaviour, these analyses have been so far applied only to the freshwater cladoceran *Daphnia pulex* (Uttieri *et al.*, 2004).

The swimming trajectories of *C. furcatus* are convoluted but compressed and limited to small regions of the available spatial domain. The tracks attain low fractal dimension values, resulting in a reduced degree of space-fillingness of the tracks and indicating that the motion occurs mainly along one direction. These results provide a quantitative confirmation of what was proposed by Mazzocchi & Paffenhöfer (1999), according to whom *C. furcatus* explores small areas of fluid in rapid succession.

From a Lagrangian point of view, the swimming trajectories of *C. furcatus* are characterised by a strong random component, as evident from the limited correlation of swimming velocities and from the values close to unity of the ratio between the turbulent and the total kinetic energies of the process. In addition, the motion is characterised by the absence of any dominant peak. This indicates that all the frequencies involved in the process sum up, and suggests that a complex hydromechanic signal might be generated. This kind of signal is probably hardly perceived by organisms in the close vicinity of the copepod.

These characteristics are independent of the occurrence of a prey capture, and the typical looping behaviour has been observed for up to 7 consecutive hours by *C. furcatus* swimming in filtered sea water. It therefore appears that the peculiar swimming motion, and probably also the parameters associated, are not affected by the availability of prey items, representing an inborn trait of *C. furcatus*.

The swimming behaviour of *C. furcatus* can be interpreted in terms of prey searching and predator avoidance strategies. The copepod performs an in-depth scanning of a confined region of fluid looking for prey items. The strong random-like characteristics of *C. furcatus*' swimming motion might be beneficial in effectively disturbing potential mechanosensitive predators. Video recordings, showing how the swimming motion of *C. furcatus* does not displace artificial lattice beads present in the fluid, provide an indirect confirmation that this peculiar behaviour does not create any recognisable wake in the environment. This peculiar swimming motion can therefore be beneficial also as a predator-avoiding strategy, even though being completely different from the classical escape responses performed by other copepods. Paffenhöfer & Knowles (1980) reported that continuously swimming nauplii are more easily detected by predators. In the case of *C. furcatus*, despite its incessant motion this small calanoid is scarcely preyed upon by *Sagitta* (Kehayias *et al.*, 1996). In this framework, as already discussed in § 2.4, the swimming motion of the copepod may elude and confuse a predator based on its peak-free spectrum and random-like characteristics, which may work as self-hiding mechanism (Hwang & Strickler, 2001).

C. furcatus captures its prey only when they are located inside its rather restricted capture area and placed frontally to its head (as sketched in Figures 3.1 and 3.2). The copepod does not attack its quarry through direct lunges, but instead seizes them via direct interception maintaining its characteristic swimming activity. The swimming and feeding behaviour thus appear closely related and mutually dependent. The fast and continuous looping behaviour does not allow the creation of a feeding current, which requires a coordinated rhythmic movement of the mouthparts that can be achieved only by smooth cruising. In addition, the typical propulsive mode of *C. furcatus* appears to hinder the copepod from rapidly reorienting towards single prey just outside the capture area.

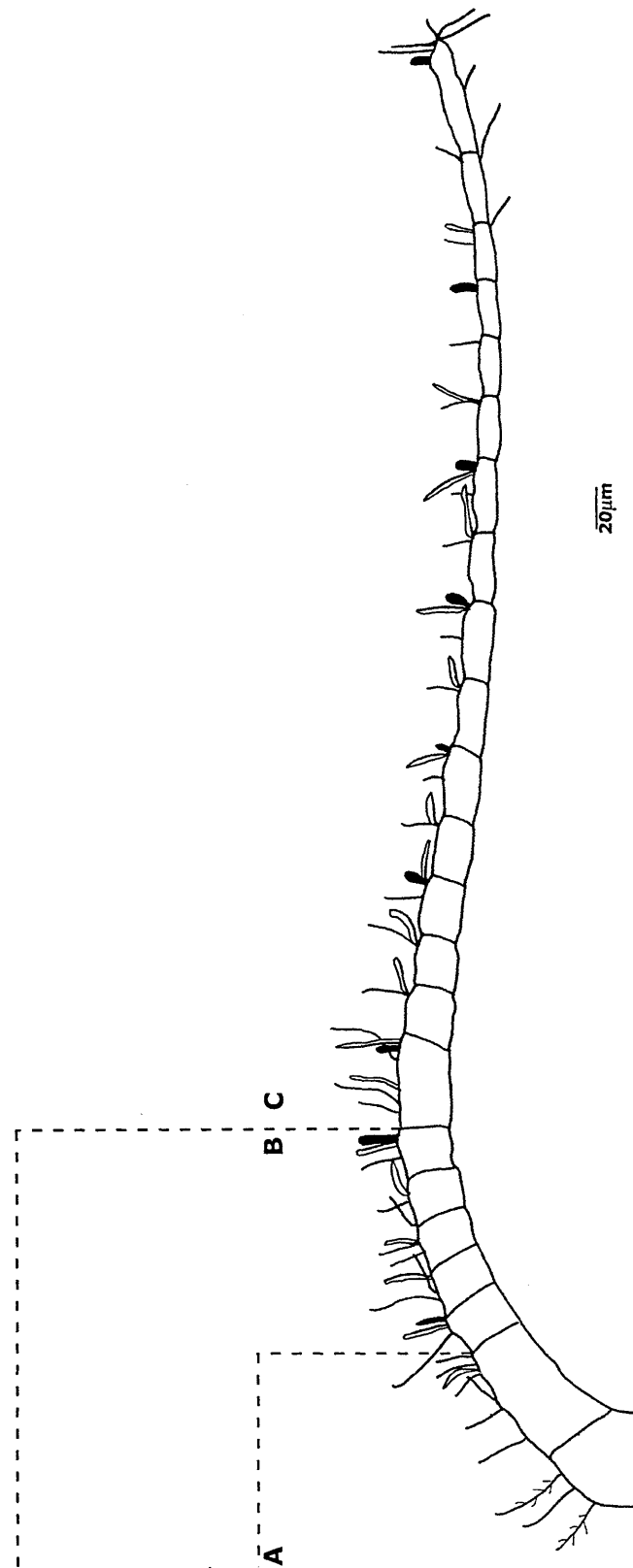


Figure 5.1: overlapping of the capture cores and of the sensory distribution along the A1 of adult female *C. furcatus*. The predatory core A coincides with segments 1 and 2, while core B corresponds to segments 3-7; from segment 8-9 onward, antennule segments are coincident with predatory core C, where no capture takes place.

The capture area of *C. furcatus* overlaps with the basal sector of the copepod's antennules (Figure 5.1). More particularly, the inner core (where the efficiency of capture EC is equal to 100%) overlaps with segments 1 and 2, whereas the medium core (EC=62%) corresponds to segments 3-7; all other segments of the first antennae are within the outer predatory core, where no capture takes place (EC=0%). This evidence shows how feeding mechanisms strongly depend on the neuro-sensory performances of the A1: captures occur only in association with the segments for which the most intense neurophysiological responses to prey-mimicking mechanical stimuli were measured. In addition, the only two serrulate setae present on the A1 are located on segment 1 and are in the inner core; this suggests that these setae might be important for enhancing the sensitivity of the basal sector of the A1 as well as for the exact detection of prey items resulting in an EC=100%. The setules present on them are likely involved in the perception of smaller scale mechanical disturbances, which might lead to a better identification of prey items.

In contrast, the other sectors of the A1 are outside the capture area, where no prey was caught: while this is rather consistent for the distal part, for which the weakest responses were measured, the situation is less clear for the median sector. In this area, in fact, the neurophysiological recordings (see Figure 4.12) showed an intermediate response between the basal and the distal sectors. The success of capture appears to be strongly constrained by the swimming motion of the copepod itself: when continuously looping at very high speed in the presence of dinoflagellate prey, *C. furcatus* seems to be unable to rapidly reorient towards a prey to attack it, even if relatively close to the base of the A1 (i.e. in the region of the median sector).

The complementarity of the outcomes emerging from the results obtained in this thesis provides a coherent overall view of the behaviour of *C. furcatus* in relation to its predatory performance. The characteristic swimming behaviour influences the predatory mechanisms of the copepod, which must rely on the collection of only those particles located almost frontally to its head. These prey are sensed by means of the

sensory structures placed on the segments of the basal part of the A1, for which the sharpest reactions to prey-like stimuli are recorded.

Somersaults are an aspect of the motion behaviour of *C. furcatus* which still remains unclear. They represent a unique feature in this species, and no reports in other species are available. In this thesis, efforts have been devoted to understanding the role played by this peculiar behaviour. Of the 77 events reported in the recordings, none is associated with a predatory event, even though Mazzocchi & Paffenhöfer (1999) reported an increase in the rate of capture proportional to the frequency of somersaulting (their Figure 7). This peculiar behaviour never occurs with a regular frequency, but indeed takes place almost randomly, even in filtered sea water (*pers. obs.*); in addition, also the number of consecutive somersaults varies without any recognisable pattern. Based on current observations, it is therefore not yet possible to provide any sound explanation for this atypical behaviour.

5.2 The “paradox” of *C. furcatus*

The peculiar traits of *C. furcatus* are not only restricted to the swimming activity (as previously discussed in Mazzocchi & Paffenhöfer, 1999), but embrace other aspects such as the prey capture strategy and the sensory performance. The integration of all these aspects depicts a scenario which, for some aspects, can be assumed as “paradoxical”.

All animals moving in a fluid have evolved specific adaptations to maximise their propulsive efficiency (Hedenström, 2004). Copepods’ streamlined body reduces the drag exerted by the surrounding environment, increasing the efficiency of motion, even if viscosity determines an increase in their metabolic requirements. Measurements of energy consumption in larger animals show that, for a given speed, an organism will adopt the motion which minimises the metabolic requirements

(McNeil Alexander, 2000, and references therein), and Blake (2000) demonstrated that aquatic vertebrates prefer intermittent swimming to continuous motion in order to save energy. Following Pyke's (1984) optimal foraging theory, an organism optimises the energy required to collect the amount of food necessary to fulfil the energetic demand associated with its own motion. Estimates of energy consumption (e.g., Vlymen, 1970; Klyashtorin & Yarzombek, 1973; Klyashtorin, 1978) and hydromechanical models of swimming movement (e.g., Haury & Weihs, 1976; Morris *et al.*, 1985 and 1990) demonstrate that copepod swimming is characterised by high efficiency but, at the same time, by considerable energetic demands that must be fulfilled through the diet.

Direct measurements of *in situ* ingestion rates and natural diet, as well as measurements of energetic costs, by *C. furcatus* are lacking. These data are fundamental for a precise estimate of the energetic costs of this species. The only information available on this topic is reported by Klekowski & Sazhina (1985), who calculated the daily cost of maintenance as per cent of body energetic equivalent. Their results indicate that *C. furcatus* is an "economic" copepod, in the sense that it has an efficient energy allocation strategy. Its continuous and fast motion however suggests that the energetic costs associated with the active metabolism are very high. Surprisingly, this latter evidence is coupled with low feeding rates (Mazzocchi & Paffenhöfer, 1998), at least in the case of homogeneously distributed prey. The copepod is successful in oligotrophic areas (e.g., Webber & Roff, 1995a; Siokou-Frangou *et al.*, 1997) and its juvenile stages show high mortality rates at high food concentration (Mazzocchi & Paffenhöfer, 1998).

The combination of all these aspects determines the "paradox" of *C. furcatus*. To properly understand the advantages and the success of its behaviour, its predatory tactics and neurophysiological performance, these aspects need to be integrated in an ecological framework.

5.2.1 The ecological framework

All organisms evolve and co-evolve together with their surroundings, developing specific adaptations (both behavioural and morphological) by which they fruitfully exploit the environmental conditions experienced. The ecological significance and adaptative advantages of the behaviour displayed by *C. furcatus* can be understood only by setting the outcomes of this thesis in the proper environmental context.

C. furcatus is a dominant species in tropical and subtropical oligotrophic regions of both hemispheres. It is present in the upper part of the water column and the core of the population is always above the thermocline (Peralba & Mazzocchi, 2004). In the Gulf of Naples, *C. furcatus* has its main peak of abundance in late summer (Mazzocchi & Ribera d'Alcalà, 1995; Peralba & Mazzocchi, 2004). During this period, the water column is strongly stratified (Ribera d'Alcalà *et al.*, 2004), a hydrodynamic condition leading to a strengthening of the pycnocline and to high Richardson (Ri) numbers; these result in a stable water column with respect to shear instabilities (Turner, 1973). Such a state is favourable for the development of thin layers of phytoplankton (Dekshenieks *et al.*, 2001; McManus *et al.*, 2003 & 2005 and references therein), which are often reported in natural conditions (e.g., Mullin & Brooks, 1976; Davis *et al.*, 1991). Phytoplankton distributions are in fact never fully homogenised, even at high levels of turbulence (Seuront, 2005), but present an intermittent pattern as a result of the intermittency of the fluid environment itself (Seuront & Schmitt, 2005a). Associated with these thin layers, are often reported higher concentrations of exudates which increase the viscosity of the medium and further damp down turbulent fluctuations (Jenkinson, 1993). In fact, below a certain critical threshold, turbulence may not be effective in disrupting phytoplankton aggregates formed by naturally-sticky species (Seuront & Schmitt, 2005b).

Phytoplankton micro-layers are characterised by at least 3 times higher concentrations of phytoplankters than in the surrounding environment (Dekshenieks *et al.*, 2001), over a vertical interval of 0.25 to 1.0 m (Cowles, 2004 and references therein). In the stratified water column in the Gulf of Naples, diatoms are dominant in

the upper 5-10 m, while flagellates and unicellular cyanobacteria spread at lower depths; the summer phytoplankton assemblage is constituted by small diatoms, phytoflagellates and dinoflagellates, whereas in autumn diatoms dominate (Ribera d'Alcalà *et al.*, 2004 and references therein).

As underlined by Seuront *et al.* (2001), predator-prey encounter rates are mainly driven by behavioural adaptations to prey patchiness. Since the probability of predator-prey encounter is dependent, among other factors, on the distribution of the two organisms involved (e.g., Lasker, 1975; Gerritsen, 1980), microlayers have a potentially significant ecological role. Recent studies show that copepods can detect aggregates chemically rather than hydromechanically (Kiørboe, 2001; Kiørboe & Thygesen, 2001). At low Reynolds numbers, viscosity rapidly attenuates mechanical disturbances, which remain detectable only for a few seconds; in contrast, chemical signals persist thanks to viscosity, which limits molecular diffusion and preserves a diffusive trail (Yen, 2000), allowing the signal to remain coherent over longer time scales than mechanical cues (Doall *et al.*, 1998; Yen *et al.*, 1998).

Following Uchima & Hirano (1988), the continuous looping swimming can be considered an optimization for the detection of micro-scale patches of food. This kind of motion, in fact, contrasts with a maximisation of the searching efficiency achieved by avoiding swimming in areas already searched (O'Brien *et al.*, 1989), resulting in the absence of path overlaps and crossovers. By scanning small volumes, *C. furcatus* can efficiently remain inside a patch once encountered, efficiently exploiting it. The numerical results in Wiggert *et al.* (2005) (their Figure 3a) provide an indirect validation of this small-volume exploration strategies. In their three-dimensional simulated trajectories, Wiggert *et al.* (2005) show that *C. furcatus* describes convoluted patterns of movement in the horizontal plane, occasionally interrupted by fast darting along the vertical direction. This behaviour recalls what observed by Tiselius (1992) for *Acartia tonsa*, which on detecting a micro-layer reduces its jump frequency to remain inside the patch. The fast darting movements observed in the

video recordings could therefore be utilised by the copepod to rapidly displace itself in the environment in search of new and unexplored patches.

The presence of aesthetascs and putative chemosensory setae along the entire antennule suggests that *C. furcatus* depends to a large extent on the detection of chemical cues from its prey. More particularly, the presence of these structures on the distal part of the A1 may serve to guide the copepod inside a far-field cluster, while those placed on the basal and median sectors might be utilised to remain inside the patch. In such circumstances, the copepod would just swim across the patch to gather food particles; in this framework, the copepod could reduce the extension of its capture area without negatively affecting its energetic requirements. It is reasonable to hypothesise that, given these conditions, *C. furcatus* might show higher ingestion rates than those measured so far (Mazzocchi & Paffenhöfer, 1998), by which it could fulfil the energetic requirements of its continuous motion.

C. furcatus therefore appears to be optimally suited for the utilisation of micropatches of food in its natural environment. This is supported by the evidence that, when the physical conditions do not sustain the formation of such micro-scale aggregates, the copepod is almost absent, lacking the physical environmental conditions to support population maintenance.

5.3 A tentative evolutionary perspective

The particular scenario developed in the previous chapters may be utilised to contextualise the behaviour of *C. furcatus* in an evolutionary perspective. The phylogenetic position of a genus or a species can in fact be revealed not only by morphological (Björnberg, 1986a and b; Huys & Boxshall, 1991) and molecular studies (Bucklin *et al.*, 2003), but also by considering behavioural aspects (Paffenhöfer, 1998).

The most commonly accepted phylogenetic tree, investigated using cladistic analysis (Ho, 1990; Huys & Boxshall, 1991; Ho 1994), considers the Platycopioidea as nearest to the ancestral copepod, soon after followed by Calanoida which, unlike the remaining orders, present a rather uniform morphology (Huys & Boxshall, 1991; Mauchline, 1998). Looking at the ontogeny of swimming activity, those copepods displaying occasional motion throughout development (e.g., *Oithona plumifera*) are considered to be phylogenetically older than those species showing active motion during ontogeny (e.g., *Temora stylifera*) (Gauld, 1966; Björnberg, 1972). These latter are considered more advanced, also in relation to the presence of feeding currents in all developmental stages (Paffenhöfer, 1998).

The only molecular investigations carried out so far on *C. furcatus* are those by Bucklin *et al.* (2003), indicating that this species is the most divergent of the genus (their Figure 1). From a swimming perspective, *C. furcatus* belongs to the second group of the most advanced species. In fact, while adult *C. furcatus* display their typical looping behaviour (Mazzocchi & Paffenhöfer, 1999), their nauplii move forwards by successive somersaults (Björnberg, 1972). However, looking at the morphology of the antennules, an opposite conclusion can be drawn. While an evolutionary trend in reduction of segments is widely accepted (Schutze *et al.*, 2000), the A1s of *C. furcatus* show a high number of expressed segments with few fusions, indicative of a primitive structure (Boxshall *et al.*, 1984). Other ancestral characters are found in the arrangement of antennular structures. Giesbrecht (as reported in Boxshall *et al.*, 1984) assumed the trithek arrangement as the typical armature of female antennules. In copepod evolution this structure has been variously modified, usually with the loss of the aesthetasc (Boxshall, 1983). The A1s of *C. furcatus* are equipped with 9 complete tritheks, distributed along the entire length of the structure. The abundance of this presumed ancestral armature supports the proximity of *C. furcatus* to the ancestral progenitor.

In this framework, it is quite difficult to assume the feeding strategy of *C. furcatus* as either phylogenetically primitive or advanced. This modality drastically differs from

feeding currents, which tend to maximise the collection of food item by means of large funnel-shaped convective flux, as well as from ambush predation, for which an enlargement of the capture area is achieved through the extensive presence of very long mechanoreceptive setae on both the antennules and the caudal rami. If feeding currents are assumed to have evolved recently, then the capture strategy adopted by *C. furcatus* can be considered as a primitive feeding mechanism.

5.4 Conclusions

It is well established how large-scale dynamics in the oceans are crucially dependent on small-scale processes. For this reason, knowing the interactions occurring at the individual scale is fundamental for understanding the functioning of pelagic systems, and in particular the role played by copepods.

Small copepods are recognised as primary actors in epipelagic communities, not only for their sheer numerical abundance but also for their impact on grazing, as well as for providing the link between lower and upper trophic levels and between the classical and the microbial food webs. The calanoid copepod *C. furcatus* is a key representative of small copepods: it is present in both tropical and subtropical waters and it is a dominant species in oligotrophic environments. Moreover, it displays a characteristic swimming behaviour, moving very fast along convoluted tracks.

Behaviour can be assumed as a trade-off between the three basic needs of a copepod: looking for prey, looking for mates, avoiding predators. As a more general definition, behaviour can be interpreted as the set of adaptations developed by a species to take advantage of the environmental conditions experienced. The driving question of this thesis was to understand how the behaviour of *C. furcatus* would sustain its success in oligotrophic environments. This question was prompted by the evidence that this species swims with a locomotory pattern which is not reported in

any other species, and that its feeding rates are low despite its almost incessant motion, to which increased metabolic requirements are generally associated.

To this aim, different approaches have been exploited and complemented with the aim of highlighting specific characteristics of different behavioural aspects (namely motion, feeding and sensitivity). The merging of these results depicts a general framework to understand which strategies and optimizations are utilised by *C. furcatus* to thrive in food diluted environments.

The results discussed in this thesis show that the kinematic properties of the motion of *C. furcatus* resemble a random process. Such strategy may result useful in not-providing distinguishable mechanical cues to either prey or predators in the close vicinity of the copepod. The rather incessant looping swimming behaviour has moreover direct effect on the mechanisms of prey capture. This characteristic motion prevents the copepod from creating a feeding current, for which a smoothly cruising and less frantic natatory behaviour is needed. While moving, *C. furcatus* can only collect those particles which are located almost frontally to its head, in a capture area which is up to ten times smaller than in other copepods. The mechanisms of prey capture are in turn dependent on the neurophysiological performances of the sensors located on copepod's antennules. The capture area, in fact, corresponds to those segments for which the most intense neurophysiological responses are recorded, whereas no capture takes place in correspondence of those segments characterised by lower-amplitude peaks or no responses in nerve impulse traffic recordings.

In which way do these peculiarities allow *C. furcatus* being so abundant in oligotrophic environments? How can such an energetically expensive swimming motion be coupled with the low feeding rates reported so far? To answer this question, the behavioural strategies emerged in this thesis can be read in terms of efficient exploitation and adaptations to the environment. The conditions supporting the peak of abundance of *C. furcatus* are the same sustaining the presence of thin-layers of phytoplankton in the water column. The swimming motion of *C. furcatus* appears to be best suited for exploiting these micro-layers of food. By describing tortuous but

restricted tracks, the copepod performs an accurate scanning of a limited area of the environment; such behaviour maximises the probability of encountering a cluster of densely aggregated particles. The continuous looping may allow the copepod to reside into the patch, thus maximising the probability of encountering and collecting particles inside its capture area. Even the shape of the capture area appears as optimised for the collection of patchily-distributed prey. In such circumstance, taking also in account the swimming behaviour of *C. furcatus*, the copepod can simply but effectively gather food particles encountered along the path, without needing any modification of its locomotory pattern. This scenario depicted for the two-dimensional case discussed in this work can be extended to the three-dimensional environment. In such circumstance, the copepod could efficiently take advantage of the thin horizontal layers of particles, using its fast oriented vertical displacements to rapidly relocate itself in new and yet unexplored regions of fluid.

The densely aggregated mechanoreceptive setae on the basal sector of the A1 allow the copepod to sense potential prey items located inside its capture area. This is supported by the recordings of intense neurophysiological responses in correspondence of the basal segments of the first antenna. By contrast, the median and the distal part of the A1 do not appear involved in the mechanical localisation of prey, but are likely utilised by the copepod to re-orient towards a patch of food. These aggregates, in fact, often release chemical exudates that could be perceived by distal chemoreceptors (aesthetascs and modified setae) of *C. furcatus*, which can therefore direct towards them. By using the chemoreceptors distributed all along the A1, the copepod can discriminate horizontal gradients of odorant molecules and consequently modify its swimming towards the patch.

Even though the results obtained in this thesis focus on the relationship between *C. furcatus* adult females and their prey, they can however provide new elements to understand the success of this species in relation to its predators. In oligotrophic environments, the abundance of living species is reduced; this goes for phytoplankton, as well as for copepods and for their own predators. It is therefore reasonable to

assume that the predatory pressure on *C. furcatus* is not very strong, even though present. However, the copepod has to overcome the skills of its predators. The frantic and incessant motion of *C. furcatus* can provide an efficient predator-avoidance strategy. The kinematic properties of its swimming behaviour in fact provide the copepod with a useful hydro-mechanical camouflage, by which to elude its predators. Such a strategy is completely different from the typical fast escape responses reported for other copepods, but it may equally represent an efficient predator-avoidance strategy.

The behaviour *sensu lato* of *C. furcatus* is optimised for an efficient exploitation of those set of environmental conditions found in the areas and in the periods concomitant to its peak of abundance, leading to its success. The results collected in this thesis provide new elements by which understanding the adaptations evolved by *C. furcatus* to thrive in oligotrophic areas of both hemispheres.

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Appendices

Appendix I

This appendix contains information, arranged in tabular form, of the sequences analysed in Chapter 2. All tracks have been recorded between 24th October and 30th November 1996.

In the present appendix, for each clip analysed the following data are provided:

- Track ID: the file name of the clip analysed;
- Female ID: the ID of the female which described the track considered;
- Track Duration (s): the length, in seconds, of the track analysed;
- # of Frames: the total number of frames considered for each clip;
- Time of Day: the period of the day in which the clip was originally recorded;
- T_x (s): integral time scale for the x-component of the motion, calculated in seconds;
- T_y (s): integral time scale for the y-component of the motion, calculated in seconds;
- EKE/TKE: the ratio between the eddy (turbulent) (EKE) and the total (TKE) kinetic energies associated with the motion;
- D: the fractal dimension of the trajectory considered;
- r^2 : the coefficient of determination associated with the least-squares regression line for D evaluation.

Swimming

Track ID	Female ID	Track Duration (s)	# of Frames	Time of Day	T _x (s)	T _y (s)	ENE/r/kE	D	r ²
swm-1	1	4.5	271	morning	0.785	0.6984	0.96219	1.0125	0.942655
swm-2	1	2	121	morning	0.4267	0.556	0.96039	1.0112	0.938589
swm-3	1	2	121	morning	0.5334	0.6121	0.99685	1.0696	0.964024
swm-4	1	2	121	morning	0.3917	0.5021	0.9716	1.0239	0.963277
swm-5	1	2.5	151	evening	0.381	0.716	0.95471	1.022	0.965474
swm-6	1	3	181	evening	0.7301	0.9523	0.97332	1.1334	0.971757
swm-7	1	3	121	evening	0.611	0.4547	0.99494	1.2324	0.986077
swm-8	1	5.5	331	night	0.8567	1.1075	0.9987	1.0553	0.957631
swm-9	1	3	181	night	0.5151	0.8666	0.99671	1.128	0.940178
swm-10	2	4	241	morning	0.7644	0.7508	0.97942	1.0597	0.960586
swm-11	2	2.5	151	morning	0.7091	0.4963	0.97496	1.1123	0.958255
swm-12	2	7	421	evening	0.9738	1.0313	0.97678	1.1386	0.962613
swm-13	2	8.5	511	evening	1.0505	1.0697	0.96495	1.1482	0.962805
swm-14	2	13.5	811	night	1.2058	1.404	0.96846	1.2085	0.960536
swm-15	2	3	181	night	0.6882	0.5218	0.97762	1.1092	0.938967
swm-16	2	4.5	271	night	0.7642	0.7786	0.96236	1.0747	0.950523
swm-17	2	3	181	night	0.6791	0.7364	0.9689	1.0128	0.933845
swm-18	3	3.5	211	morning	0.578	0.7881	0.98012	1.0559	0.951657
swm-19	3	6.5	391	morning	0.7333	1.0801	0.9742	1.1306	0.958489
swm-20	3	4	241	morning	0.7069	0.689	0.97619	1.0975	0.963001
swm-21	3	10.5	631	morning	1.4102	1.1823	0.97907	1.2426	0.9681
swm-22	3	2.5	151	evening	0.8369	0.5614	0.96635	1.0434	0.962052
swm-23	3	7	421	night	1.0347	0.9263	0.97167	1.2423	0.97378
swm-24	3	3	181	night	0.6116	0.779	0.98222	1.0974	0.978857
swm-25	3	2.4	148	night	0.3346	0.3791	0.98209	1.0667	0.937649
swm-26	3	3	183	night	0.7006	0.8071	0.99629	1.0294	0.978802

sww-27	3	5	301	night	0.7158	0.8694	0.97485	1.1538	0.957782
sww-28	4	2.5	151	morning	0.8941	0.5805	0.95833	1.1238	0.950466
sww-29	4	9.5	571	morning	1.0191	1.7014	0.98972	1.1779	0.950716
sww-30	4	2.5	149	morning	0.6973	0.6374	0.95109	1.0337	0.951469
sww-31	4	3	181	evening	0.4604	0.5064	0.98918	1.0384	0.951872
sww-32	4	2.5	151	evening	0.4532	0.9016	0.97306	1.0194	0.960936
sww-33	4	5.5	331	evening	0.7955	0.8433	0.98749	1.2394	0.976797
sww-34	4	5.5	331	evening	0.9775	0.7363	0.97852	1.1257	0.973877
sww-35	4	5.5	331	night	1.6074	0.77	0.99007	1.0709	0.948552
sww-36	4	5	301	night	0.8612	0.7436	0.94742	1.1014	0.957222
sww-37	5	4	241	morning	0.6052	0.8941	0.94318	1.0194	0.939568
sww-38	5	9	541	evening	0.9154	1.1199	0.97619	1.174	0.953422
sww-39	5	7	421	evening	0.8181	1.786	0.97775	1.1848	0.975094
sww-40	5	18	1081	evening	1.0945	1.4931	0.96606	1.2641	0.964366
sww-41	5	11.5	691	evening	1.1139	1.1716	0.97912	1.246	0.969164
sww-42	5	3	181	night	0.3657	0.3538	0.94839	1.0464	0.967703
sww-43	5	3	181	night	0.5322	0.5273	0.96489	1.0089	0.971702
sww-44	5	4.5	271	night	0.8333	0.7885	0.95758	1.0569	0.960034
sww-45	6	3.5	211	morning	0.5677	0.9433	0.95272	1.0283	0.944339
sww-46	6	3.5	211	morning	0.6473	0.5804	0.97794	1.037	0.977867
sww-47	6	4.5	271	evening	0.6586	0.9255	0.96626	1.1288	0.954558
sww-48	6	3.5	211	night	0.62	0.5103	0.97871	1.0328	0.979305
sww-49	6	7	421	night	0.816	1.0464	0.9827	1.1232	0.958708
sww-50	6	4.5	271	night	0.7839	0.6993	0.97588	1.0125	0.942655
sww-51	7	2	120	evening	0.0359	0.04	0.98893	1.0334	0.964391
sww-52	7	4.8	291	evening	0.0792	0.0803	0.96178	1.0733	0.95061
sww-53	7	3.2	192	evening	0.0571	0.0517	0.96423	1.0264	0.968844
sww-54	7	7	421	evening	0.1044	0.1008	0.98375	1.1889	0.965459
sww-55	8	5.8	350	morning	0.1364	0.0992	0.99282	1.3753	0.981279
sww-56	8	2.4	147	morning	0.0596	0.0456	0.94903	1.0949	0.950867

Capture

Track ID	Female ID	Track Duration (s)	# of Frames	Time of Day	T _A (s)	T _V (s)	ENE/Hz	D	P ²
cpt-1	1	6.5	391	morning	1.5312	0.9586	0.99775	1.1802	0.983773
cpt-2	1	4	241	morning	0.737	0.5684	0.96722	1.0133	0.960744
cpt-3	1	2	121	morning	0.4356	0.8064	0.97195	1.0494	0.97226
cpt-4	1	3	181	evening	0.8969	0.6831	0.98526	1.1472	0.954139
cpt-5	1	5	301	evening	0.9341	0.9701	0.97343	1.2222	0.974267
cpt-6	1	2	121	night	0.5738	0.6134	0.99179	1.0938	0.95289
cpt-7	2	2.7	161	morning	0.571	0.6232	0.99034	1.1116	0.96584
cpt-8	2	4	241	morning	0.7345	1.1421	0.96644	1.0695	0.956015
cpt-9	2	2	121	morning	0.5663	0.5756	0.97051	1.0561	0.960256
cpt-10	2	3	181	morning	0.8746	0.6122	0.99385	1.141	0.976961
cpt-11	2	7	421	evening	0.8494	1.0286	0.97979	1.317	0.985462
cpt-12	2	3	181	night	0.6179	0.5039	0.97946	1.1104	0.986984
cpt-13	3	2.4	144	morning	0.7821	0.6032	0.96059	1.2348	0.964367
cpt-14	3	4.9	295	morning	0.8103	0.7511	0.99519	1.1458	0.975207
cpt-15	3	4	241	evening	0.5739	0.8124	0.98311	1.1795	0.947707
cpt-16	3	8.3	497	evening	1.0746	1.0277	0.95544	1.2225	0.983062
cpt-17	3	5	301	evening	0.9431	0.9561	0.99769	1.2409	0.972556
cpt-18	3	2.9	175	night	0.3844	0.7461	0.94274	1.3456	0.946953
cpt-19	3	5	301	night	0.888	0.8875	0.95824	1.1463	0.968849
cpt-20	3	2	121	night	0.5715	0.6503	0.97916	1.0689	0.951301
cpt-21	4	2	121	morning	0.6752	0.3943	0.99433	1.0574	0.966153
cpt-22	4	3	181	morning	0.5779	0.6474	0.98359	1.1353	0.976507
cpt-23	4	4	241	morning	0.7294	0.6826	0.98629	1.2056	0.980314
cpt-24	4	4	241	evening	0.7428	0.8172	0.97083	1.0843	0.968689
cpt-25	4	2	121	evening	0.4234	0.5542	0.98836	1.0515	0.973727
cpt-26	4	5	301	evening	1.0396	0.7615	0.96902	1.1515	0.971931

cpt-27	5	2	121	morning	0.4055	0.3736	0.92564	1.2346	0.93427
cpt-28	5	3	181	evening	0.749	0.6978	0.98022	1.1356	0.944604
cpt-29	5	3	181	evening	0.8198	0.5565	0.98811	1.1115	0.992679
cpt-30	5	3	181	night	0.5122	0.66	0.98072	1.0109	0.947738
cpt-31	6	4	241	morning	0.8751	0.6416	0.99369	1.2032	0.968583
cpt-32	6	7.3	440	night	0.9083	0.9544	0.97219	1.2157	0.977507
cpt-33	6	2	121	night	0.4961	0.5147	0.99829	1.0217	0.985483
cpt-34	6	7	421	night	0.9837	1.2547	0.99034	1.1663	0.966029
cpt-35	7	5	301	evening	0.1094	0.0852	0.95935	1.0643	0.936147
cpt-36	8	8	483	morning	0.0859	0.1036	0.96628	1.1495	0.954593

Appendix II

This appendix contains information, arranged in tabular form, of the sequences analysed in Chapter 3. More particularly, for each clip analysed, the following data are provided:

- Track ID: the file name of the clip analysed;
- Female ID: the ID of the female which described the track considered;
- Track Duration (s): the length, in seconds, of the track analysed;
- # of Frames: the total number of frames considered for each clip;
- Time of Day: the period of the day in which the clip was originally recorded;
- # of prey visible in the clip: the total number of dinoflagellates contemporary present in the clip considered and in focus with the copepod;
- # of prey items captured: the total number of dinoflagellates captured (if any) in the clip considered.

Track ID	Female ID	Track Duration (s)	# of Frames	Time of Day	# of prey visible in the clip	# of prey items captured
clip-1	1	0.4	26	morning	2	0
clip-2	1	1	64	morning	5	0
clip-3	1	2.5	147	morning	3	1
clip-4	1	1	65	morning	2	1
clip-5	1	1	61	morning	2	0
clip-6	1	1	58	evening	1	0
clip-7	1	1.2	76	evening	1	0
clip-8	2	0.7	43	morning	1	1
clip-9	2	1.2	73	morning	3	0
clip-10	2	0.7	42	evening	2	1
clip-11	2	1.8	109	evening	2	1
clip-12	2	1	59	evening	2	1
clip-13	2	1.1	69	evening	2	1
clip-14	2	0.6	35	night	6	1
clip-15	2	0.7	42	night	2	1
clip-16	3	1	59	morning	2	1
clip-17	3	2.1	128	morning	3	1
clip-18	3	1.1	67	evening	3	0
clip-19	3	1.3	79	evening	3	1
clip-20	3	1.6	97	evening	1	1
clip-21	3	1.2	74	night	1	1
clip-22	3	1	58	night	2	1
clip-23	3	1.1	68	night	1	1
clip-24	3	1.6	95	night	1	1
clip-25	3	1.5	91	night	1	1
clip-26	3	1.2	71	night	2	1
clip-27	4	0.9	52	morning	1	1
clip-28	4	0.8	49	morning	2	0

clip-29	4	0.8	49	morning	1	1
clip-30	4	0.6	36	morning	1	1
clip-31	4	0.8	49	evening	1	1
clip-32	4	1	63	night	1	1
clip-33	4	1.2	71	night	2	1
clip-34	4	0.6	38	night	1	1
clip-35	5	0.7	45	morning	1	1
clip-36	5	0.5	29	morning	1	1
clip-37	5	1.1	65	evening	4	1
clip-38	5	2	118	evening	3	1
clip-39	5	1.3	77	evening	3	1
clip-40	5	1.4	85	evening	3	1
clip-41	5	1.1	69	evening	2	1
clip-42	6	0.6	34	night	2	1
clip-43	6	0.9	55	morning	2	1
clip-44	6	1.2	73	morning	3	1
clip-45	6	0.7	42	morning	2	1
clip-46	6	1	57	morning	4	0
clip-47	6	0.9	55	morning	1	1
clip-48	6	0.6	37	evening	1	1
clip-49	6	0.5	30	evening	1	1
clip-50	6	1.6	98	evening	1	1
clip-51	6	1.8	111	evening	0	0
clip-52	6	1.3	79	evening	1	1
clip-53	7	1.8	112	night	1	1
				evening	1	0

